THE NATURE OF BIOLOGICAL SYSTEMS AS REVEALED BY THERMAL METHODS

Hot Topics in Thermal Analysis and Calorimetry

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The Nature of Biological Systems as Revealed by Thermal Methods

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Contents

Preface

After a kind motivation by Judit Simon (Editor-in-Chief of the *Journal of Thermal Analysis and Calorimetry*, Kluwer Academic Publisher) and negotiations with possible contributors – lasting for more than one year – it was decided to write a book about the application of thermal methods in biology. Its aim was to be a guide how to perform experiments and what kind of information might be gained by them. We tried to collect information that could be achieved only during a long personal practice. In this way scientists from biology and medicine , e.g., who are not so skilled in physics and mathematics may realize very soon the beauty and power of this tool at one hand. On the other hand, those scientists with better background in natural sciences can be more sensitive to find out exciting biological problems.

The recent situation in the literature of thermal methods (as techniques) and their application to biological problems is such that there are plenty of monographs discussing the working principles of different types of thermal analysis and calorimetry. Such books mainly deal with the general principles and present applications typical for inorganic materials. Moreover, there are some good, but relatively old reviews from the field of food physics and from different sections of biology. But it is known that the 'devil is hidden in the details': therefore, a beginner in the field of biological thermal analysis or calorimetry should 'find out' everything by his own when the principles of thermodynamics are tried to be applied to biological systems. These are highly organized and very complex objects where water and the different types of weak interactions among the macromolecules (dipoles, H-bonds, van der Waals forces etc.) make the interpretation of thermal events rather difficult.

After many discussions with colleagues at various international conferences during the last one and half year I do hope that our book will find an interested acceptance in the bio-community due to the choice of topics and authors. Moreover, the following reasons support my expectation:

- Biological calorimetry and of course thermal analysis find an increasing interest in the natural sciences community also, but both are still stepmother like treated in textbooks, monographs and journals.
- The spectrum of the book is rather broad, expanding from polymers and food over tissues to whole organisms in their active state.
- It presents macroscopic methods for rather inhomogeneous material where micromethods are often impossible or senseless.
- Thermal analysis as well as calorimetry are non-invasive and impose only limited or even no restrictions at all on the systems under research.

- The book may stimulate corresponding research and perhaps establish better contacts between very distant fields like Food Industry and Medicine, e.g.

We do not know of any book with such an orientation in the field of thermal analysis applied to life sciences.

The scientific problems discussed in this monograph are organized in four parts.

Part I. renders an insight into the properties of biotechnological polysaccharides combining the information from experimental data of thermoanalytical origin with that from statistical-thermodynamic models. Foods are discussed as multi-component and multi-phase systems where the heat treatment can produce transitions of compounds from one phase to another. Ingredients, starch-based biodegradable polymers, have an influence on the texture of the product, and thus the glass transition phenomena should be taken into consideration in the processing techniques. Proteins and fats are involved in the formulation of many food emulsions. Their structure and concentration have effects on the physical stability and organoleptic quality of emulsions while heating and cooling steps during the processing influence the storage quality.

Part II. presents examples how to use Differential Scanning Calorimetry (DSC) for structural and functional studies of muscle proteins. An exciting field of muscle research is discussed from different motor or regulator proteins up to highly organised muscle fibres. The cyclic interaction of myosin heads with actin filaments fuelled by ATP hydrolysis is basis of molecular mechanism of a number of events in biological motility. One may find studies on nucleotide-induced structural changes in the myosin head and in actin, simulating the different intermediate states of ATP hydrolysis. Interaction of F-actin with myosin heads, tropomyosin and other actin binding proteins serves as an example of studies on protein–protein interactions. Combination of DSC with other methods (e.g. electron paramagnetic resonance spectroscopy (EPR)) renders the molecular dynamic interpretation of global structural changes.

Part III. contains a review from the field of plant and plant tissues, thermobiochemical studies of animal cells in vitro, thermal investigations of social insects and an introduction into the world of human cartilage from the point of view of arthritis and degenerated lumbar intervertebral discs. We will see that wood as one of the most important plant products opens a new field for application of thermal analysis. Insects themselves represent more than half of the animal biomass on Earth so that their energetic impact can not be underestimated. Their energy saving e.g. by insulation of wasp nests or by the bee cluster strategy for surviving at low temperatures are also exciting thermoanalytical problems.

Part IV. demonstrates some efforts to make thermal analysis more quantitative by application of technical and theoretical improvements. The experimental heat capacity of carbohydrate–water systems is explained in terms of their molecular motion. Such an approach should also be valid for a more realistic description of

heat capacities of other biological materials, including cellulose–water or protein–water systems.

A new result in connection with thermal stability of proteins is that in the statistical mechanical analysis a simple transformation following the Gibbs-Helmholtz equation $G = H - TS$ is no good approximation around the transition temperature. This suggests that the thermal transition of protein molecules is actually a phase transition. Therefore, in a correct statistical mechanical analysis the system should be deconvoluted into several thermodynamic states that satisfy the necessary condition for the Legendre transformation.

This short introduction to the content of this monograph shall just bring the reader to his favourite topic on a short way. Authors and Editor will be happy to receive comments, criticism and remarks in connection with this book to improve its quality for a possible next edition in future.

As the Scientific Editor of this volume and author of some chapters, I would like to thank all the staff of the *Journal of Thermal Analysis and Calorimetry* for the help, which was given to me during the technical editing of this book.

Dénes Lőrinczy, **Editor**
December of 2003, Pécs (Hungary)

Chapter 1

Order-disorder conformational transitions of carbohydrate polymers

The calorimetry contribution to understand polysaccharide solution properties

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Introduction

Polysaccharides have often been treated as *'the poor relations'* in comparison with other highly important biopolymers, nucleic acids and proteins. It is not yet clear whether this axiom was, at least in the past, generated by the conviction/belief that the application of quantitative methods of structural, functional and biological investigations could only seldom be used. As a consequence, the studies of physico-chemical properties and their interpretation seem to have been limited, much more than would have been expected in view of the intrinsic peculiarity of the complex chemical structure of many polysaccharides.

Among these limitations, we would like to focus here on the use of thermodynamic approaches that are very well established for the characterisation of the 'molecular domains' of biomacromolecules, and which are relevant for the energetics and the structural organisation, let us say, of globular proteins (for example, Privalov, 1980a, b). It is never adequately appreciated that thermodynamics, while not providing any information on the detailed structural organisation of molecules, does infact give a body of mathematical correlations between all the properties of the system and is therefore able to identify, from among several models, the one(s) compatible with the observed experimental data. Modelling of macroscopic rheological behaviour (e.g. for physical gels) is one of the most appealing aims. It is therefore rather surprising to notice that literature tends to gloss over the correct use of thermodynamic tools or even provides a misinterpretation of the calorimetric determination of the enthalpy of the helix-coil transition in polysaccharides. This fact prompts us to clarify how much can be gleaned from

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the data collected on this transition. For this to be done, some original data produced in the authors' laboratory are presented together with a review of data obtained from literature. The aim is to instigate a full analysis of calorimetric data on the conformational transition of polysaccharides in order to provide relevant information on structural parameters that are not easily determined otherwise.

Theory

Many biopolymers undergo conformational transitions as a function of composition and/or temperature. Conformational transitions are *'in themselves'* conceptually analysed as phase transitions, since the polymer state is characterised by a difference in the structural and thermodynamic properties. We shall briefly summarise the experimental results which can be obtained by differential scanning calorimetry on the helix ↔ coil (in some cases including gel ↔ sol) conformational transition in linear biopolymer chains.

Before analysing some of these conformational transitions which occur in ordered polysaccharides, let us briefly recall some underlying concepts which have dealt mainly with the helix ↔ coil 'phase' transition of polypeptides and proteins (Poland, 1978; Cantor and Schimmel, 1980). In the case of globular proteins, it is worth mentioning the original observation that the contribution of the individual aminoacids to the Gibbs free energy of the native species is often about 200–600 J/mol of aminoacid (2–6 J/g) lower than for the denatured random coil form. Due to this low free energy difference of the monomers, the temperature of transition between the two species is higher than the ambient temperature (i.e., $T_m > 25°C$) only if the whole macromolecule can be thermodynamically considered a single domain, a fact that brings the total free energy difference to the order of 40–60 kJ per mole of protein. The hypothesis was therefore made that denaturation is a 'cooperative' process between two distinct states, which are thermodynamically defined and in equilibrium with each other at the transition temperature. The confirmation of the validity of this hypothesis, by means of DSC, represented one of the most significant milestones in the thermodynamics of biopolymer systems. It is also useful to make a reference to the current understanding of the peculiar thermodynamic behaviour of nanostructured systems which have nowadays an increasing relevance. The dependence of the melting temperature on the dimension of polymeric crystals at nanometers size is theoretically predicted by the Thompson-Gibbs equation and experimentally known since long time (Keller *et al.* 1993); crystalline lamellae show melting temperatures linearly decreasing with the inverse of the lamella thickness in the nanoscale. Similarly, for isolated polypeptide chains the helical stability (in helicogenic solvents) is predicted to asymptotically increase with chain length. Figure 1 shows the chain length dependence of the polypeptide helix-coil transition temperature (adapted from Cantor and Schimmel) and in the same plot the melting temperature (mirror-scale) of polyethylene lamellae as a function of the number of methylene units in the chain thickness.

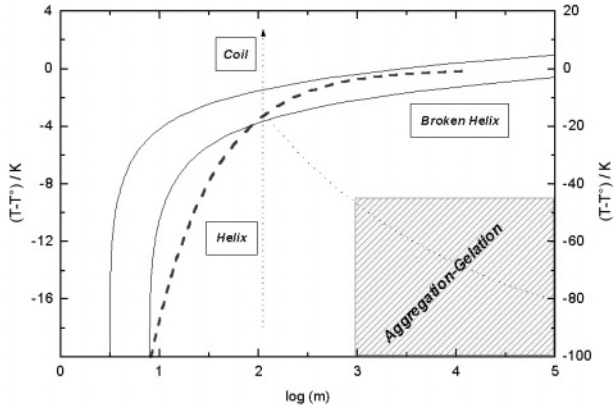


Fig. 1 Generalised phase diagram of transition temperature (arbitrary scale) for the helix-coil transition as a function of logarithm of degree of polymerisation *m* (adapted from Cantor and Schimmel). Regions for coil, helix, broken helix and aggregation state are shown. Dotted curve show the dependence of melting temperature (arbitrary scale) of paraffin-polyethylene system

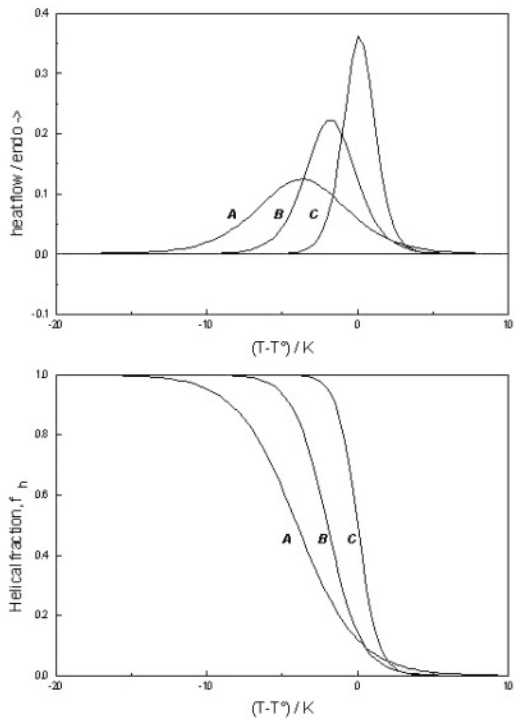


Fig. 2 Schematic DSC curves and dependence of helical fraction as a function of temperature for the three cases with increasing cooperativity (from A to C)

Order-disorder (melting) transition of helical conformations can be therefore traced by heating scans in a calorimeter (Fig. 2). The complete statistical thermodynamic description of the heat capacity curve provided by a DSC experiment showed that not only can all the information on the transition be obtained directly from the analysis of the shape of these curves without any additional data being used (Freire and Biltonen, 1978), but also that the resolution of the intrinsic structural energetics of the biopolymer and ligand binding interaction is possible by a global linkage analysis of two-dimensional DSC (Straume and Freire, 1992). This is due to the fact that the thermodynamic value of the enthalpy of the process can be written as

$$\langle \Delta H \rangle = \sum_{i-1}^{n} \Delta H_i \left(\frac{1}{Q} \right) \exp\left(-\Delta G_i / RT \right) \qquad (1)$$

by means of the elementary enthalpy ΔH_i and the probability of each step (given by Gibbs energy difference ΔG_i and the partition function Q). While the reader is referred to the ample literature, the following paragraphs outline a summary of the fundamental concepts and equations for the practical, simple, use of the experimental DSC data on the helix-coil transition.

THERMODYNAMIC STABILITY AND HELIX-COIL TRANSITION IN BIOPOLYMERS

Whenever biopolymers have a regular sequence of units, the stability of ordered helical structures is also a function of chain-length with a critical value above which the helix is interrupted (Poland, 1978). This concept was introduced, before the above mentioned findings for globular proteins, by the Zimm-Bragg theory (Zimm and Bragg, 1959) by means of the cooperativity parameter σ. This parameter essentially defines the excess free energy of formation of an isolated helical conformation with respect to the same process occurring as a neighbour of a helical sequence, for which the free energy change associated is described by the parameter s. Terms like 'initiation' and 'propagation' of a cooperative helical conformation were then suggested. The σ parameter is related to the sharpness of the change in any property measured as a function of a variable inducing helix-coil transition.

The original statistical-mechanical matrix model developed for the helix-coil transition in linear polypeptides has already been generalised to include other parallel phenomena such as, for example, the zippering of ordered chains in double or triple helices (Poland, 1978; Cantor and Schimmel, 1980). It has been also used to treat the binding of small iodine molecules into the amylose core that effectively induce the ordering conformational transition (Cesàro *et al.* 1986). In these theoretical approaches, the partition function is written as $Q = \underline{P}\,\underline{U}^m\,\underline{Q}$, where the statistical weight matrix $\underline{U}$ is properly indexed for every nearest-neighbour interaction on the polymer of chain-length m, each element in the

matrix giving the relative probability (statistical weight) for finding site i ($1 < i < m$) in a particular state, helical (h) or coil (c). Proper differentiation of the partition function with respect to the statistical weights give the thermodynamically averaged quantities which characterise the helical features of the chain in terms of the average number of helical segments, $\langle N_h \rangle$, and the average number of monomers in a helical segment, $\langle N^\circ \rangle$, defined by:

$$\langle N_h \rangle = \mathrm{dln}Q / \mathrm{dln}s \quad \text{and} \quad \langle N^\circ \rangle = \mathrm{dln}Q / \mathrm{dln}\sigma \tag{2}$$

These two quantities are sufficient to model the long polymer chain into few or several helical segments of defined length according to the value of σ (smaller σ fewer the number of broken helix, see Fig. 3).

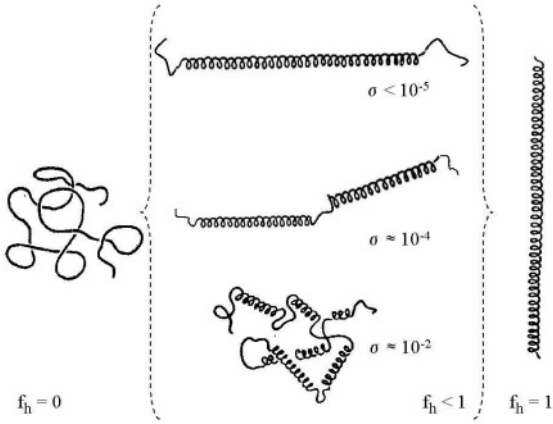


Fig. 3 Representation of dimensional (conformational) properties of chains undergoing coil-to-helix transition with different cooperativity

Without going into further detail of these theoretical approaches (Poland, 1978; Cantor and Schimmel, 1980), the prediction is that the cooperativity of the transition depends on the parameter σ, but also on the chain-length m, whilst the average transition temperature depends on m and mainly on the value of s. To underline the role of the chain length on the stability, let us remind that the phase diagram reported in Fig. 1 shows not only the stability of ordered helical conformations, but also the breadth of the transition, as a function of the variables m and T (for fixed values of σ and s).

It is also important to recognise the consequences that changes in the value of the parameter σ have in the dimensional properties (and in all other properties related to chain topology, e.g. rheology). This correlation has been theoretically clarified by Flory and co-workers (Flory, 1969), by calculating the chain dimensions (radius of gyration) of polypeptides with different cooperativity as a function of the helical fraction (related to s). Figure 4 shows the relative dimensional changes of an idealised polymer chain as a function of the helix fraction f_h for

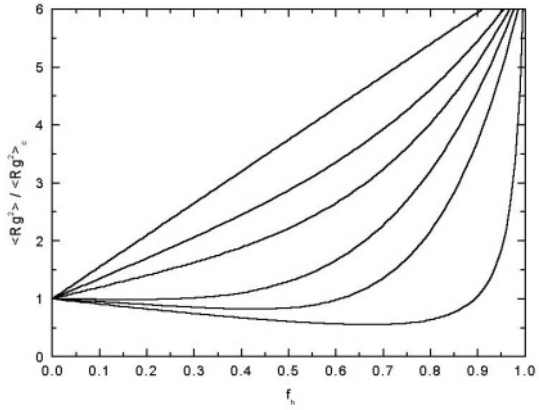


Fig. 4 Relative changes of dimensional properties (given by the square radius of gyration) as a function of the helical fraction for different values of cooperativity. The cooperativity parameter σ changes from 1 to 10^{-5} from top to bottom

different cooperativity values. Once more, the non-linear change (very abrupt, for $\sigma \approx 10^{-3}$ or smaller) emphasises the influence of the cooperativity on other physical properties, a matter of great importance for both the scientific implications and the technological applications. It is surely intriguing to note that the radius of gyration of a polymer is intrinsically related to its dynamical properties and that there is a linear log-log dependence of the average chain correlation time with the chain dimension (Cesàro *et al.* 2002). As a conceptual speculation, the increasing rapidity of helical chain collapse as a function of cooperativity closely reminds the phenomenon of fragility of supercooled liquids in a scaled Arrhenius plot of temperature dependence of segmental relaxation times (Angell, 1997). Long range correlation in fragile liquids and in cooperative helical chains are the key-parameters that will have to be further analysed in order to explore the usefulness of this conceptual correlation.

THEORETICAL ANALYSIS OF MICROCALORIMETRIC DATA

Since the earliest experiments (for example, the 'denaturation' of poly-γ-benzyl-L-glutamate, Ackermann; 1969), DSC experiments have always been more frequently used to characterise the helix-coil transition process in biopolymers. It was immediately noted that the heat of transition evaluated from DSC experiments differs from that evaluated by using the van't Hoff isochore for the apparent equilibrium constant. This discrepancy is a direct consequence of, and theoretically related to, the existence of 'molecular blocks of monomer units' which undergo a phase transition, with a change in enthalpy which is greater than the unitary change (i.e., per residue) by a factor of $\sigma^{-1/2} \approx N°$, which has been defined as the number of monomer units in a cooperative segment. Calorimetric measurements directly provide the value of $N°$ as the ratio of the apparent van't Hoff heat

of transition and the calorimetric one. Simplistically speaking, this is also the reason why, although the specific heat of fusion of ice is 1.436 kcal/mol, a van't Hoff analysis of the temperature dependence of, let us say, the density in the melting region would provide an 'apparent' heat of fusion that is higher by very many orders of magnitude, given the size of the thermodynamic domains (crystals) undergoing the transition.

The most simplified approach gives the length of the cooperative unit in terms of the specific *excess* heat capacity of the system at the transition mid-point T_m and of the specific enthalpy change for the transition Δh:

$$\frac{\Delta H^{\mathrm{vH}}}{\Delta H^{\mathrm{cal}}} = \frac{4RT_{\mathrm{Tm}}^2 \Delta c_{\mathrm{p}}^{\mathrm{Tm}}}{\Delta h^2} = N^\circ \tag{3}$$

where ΔH^{vH} is the van't Hoff enthalpy of the 'equilibrium process', defined in terms of the partition function Q:

$$\Delta H^{\mathrm{vH}} = RT^2 \frac{\partial \ln Q}{\partial T} \approx RT^2 \frac{\mathrm{d} \ln K}{\mathrm{d}T} \tag{4}$$

where K is the 'a-dimensional' equilibrium constant of the process which, according to the measurement method, can only be defined by the fraction of the species in the state h or in the state c, f_{h} and f_{c}. That is, the equilibrium constant, $K = f_{\mathrm{i}} / (1 - f_{\mathrm{i}})$, is expressed through any experimental value sensitive to the molecular state of the system, such as the intensity of the absorption, the dichroic or fluorescence band, as well as structural and thermodynamic properties.

In all cases, K is defined as a fractional ratio of the final state to the initial state. The definition of the molecular size of the species undergoing the transition is a consequence of the statistical mechanical analysis of the 'cooperativity' of the process. In other words, because of the definition of the equilibrium constant, the molecular weight enters the van't Hoff equation only for the determination of the enthalpy change involved in the process.

From the structural point of view the regularity of primary structure involves the possibility that the chains with ordered helical conformations may form supramolecular structures, either of single or multiple strand type. However, it must be noted that the above mentioned analysis does not give the number of chains involved in the helical domain, but only the average number of monomers in the domain. Nonetheless, it has been shown that, in some cases, it is possible to use the concentration variable as an additional parameter to reveal such a further stage of helix dimerisation or multiple aggregation. Theoretical work on some of these processes has been published (Poland, 1978; Kidokoro and Wada, 1987; Robert *et al.* 1989). Complex transitions can be analysed within the framework of the polysteric model for conformational transitions, as has already been done for the polysaccharide succinoglycan (Burova *et al.* 1996). Although this is very important for many hydrocolloids, we are not

considering here the statistical mechanical analysis that can be carried out on biopolymers which, in addition to the helix-coil transition, exhibit these further associations of helical segments in larger aggregates and/or supramolecular structures. Therefore, at this level of interpretation, we wish to underline that, while the stability and size of the thermodynamic domains are clearly defined through the DSC experiments, the actual molecularity of the process may still need to be supported in the model by other evidence. Past investigators resort to interpretation of experimental data, such as those given by light-scattering determination of the 'mass per unit length', or poly-electrolytic assessment of the 'charge per unit length' in the case of charged biopolymers. Recent exploitation of non-contact atomic force microscopy (AFM) in the tapping mode to solvated biopolymers opens a new avenue for direct access to molecular conformational data.

SOME RECENT DISPUTE ON THE VAN'T HOFF ENTHALPY

Only the fundamental aspects have been reported of the theoretical background which accompanied the development of calorimetric analysis of the cooperative conformational transitions of biopolymers and of discrepancy between van't Hoff and calorimetric enthalpies. However, at the end of this brief outline it seems more than appropriate to comment on some recent dispute about this question. Argumentation and rumours schematically concern two problems: *i*) the presence of small heat capacity changes that, even if not clearly discerned, induce discrepancies between ΔH_{vH} and ΔH_{cal}: *ii*) the possible intrinsic discrepancy of the two-state model. To be clear since the very beginning, none of the major criticisms and argumentation refers to biopolymer cooperativity to a first instance.

The original thermodynamic revisitation of the van't Hoff assumptions was made about ten years ago (Weber, 1996). Literature rejections was almost immediate and his argumentation was thereafter shown to have been unproperly developed as 'basic premise of his argument was incorrect, generating results fatally flawed' (Holtzer, 1997; Ragone, 1995). However, the dispute returned the question of the presence of hidden contribution to the data cast in the form of van't Hoff plot due to small values of ΔC_p. The discrepancies between the two calculated values of enthalpy, ΔH_{vH} and Δh_{cal}, are originated by different factors illustrated by several authors.

First of all, a non-vanishing heat capacity change ΔC_p introduces a curvature of the van't Hoff plot. For some binding reactions Sturtevant and coworkers (Liu *et al.* 1995, 1997; Naghibi, 1997) calculated temperature dependent ΔH_{vH} values which differ from those calorimetrically obtained; the ratio of $\Delta h_{cal}/\Delta H_{vH}$ varied from ca 0.5 to 4.3. Although a clear explanation was not provided, the indication was given that ΔH_{cal} includes all contributions from any processes underlying the reaction (including buffer or solution components) while ΔH_{vH} refers to the given 'simple' equilibrium. In a successive paper by the same authors, 'the discouraging conclusion' was reached that chemical reactions, at least in solution, are quite generally more complex than indicated by the simple

chemical equations. It has been also analysed the question of whether or not the differences arise from real underlying physical reasons, or from 'more mundane' difficulties in the proper analysis of the van't Hoff data. The effect of hidden contribution, arising from small ΔC_p values into the van't Hoff analysis, may bias the slope even if apparent curvature is not produced. On the other hand, good calorimetric and van't Hoff data might be used to infer the existence of a ΔC_p small in magnitude. Let us also explicitly mention that the differences are often more illusory than real.

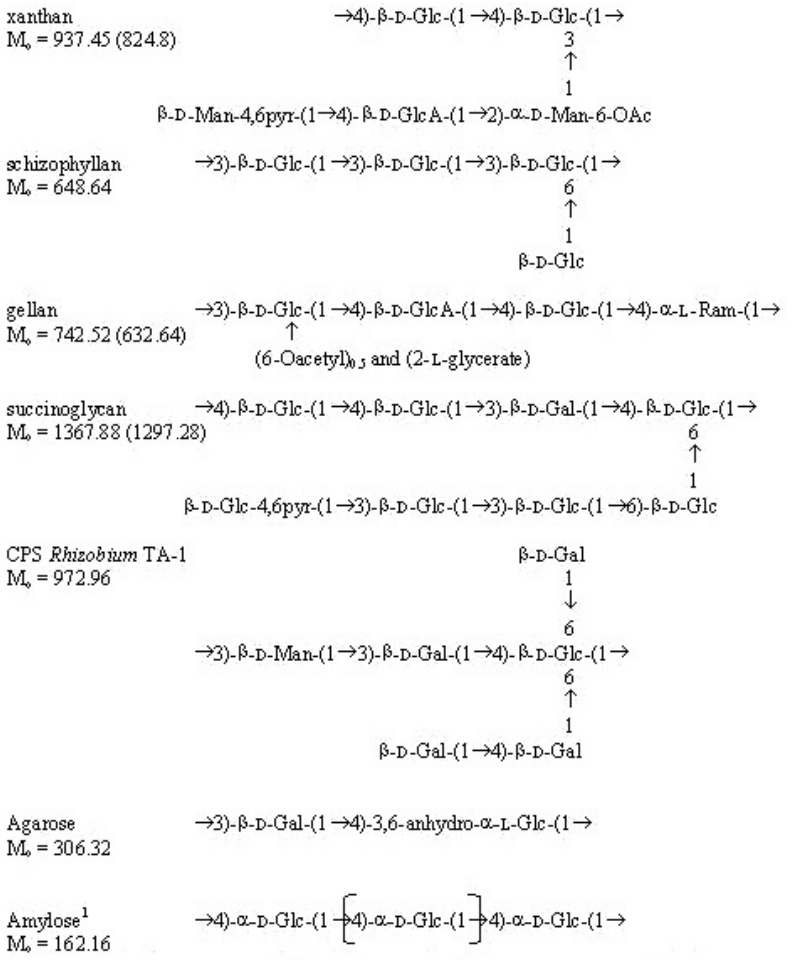
The main reason to have reported the above comments is dictated by the necessity of completely differentiating between doubts and argumentation about possible discrepancies and the 'real' large differences that are found when 'nanosize-organised' systems are disrupted by temperature and their decomposition is studied by calorimetry or followed by measuring the change in any composition-dependent properties. In the latter case, the very large values of ΔH_{vH} are uniquely, although not precisely, interpreted in terms of collapse of the macrostructure involving a large number of molecular units, while calorimetric output can be normalised by any arbitrary unit amount (generally weight or mole of substance). The term cooperativity unambiguously defines the melting of finite nano-ordered species as well as the disordering of linear Ising chains (Zimm and Bragg, 1959).

Differential scanning microcalorimetry

Several high sensitivity instruments are available from different producers. In a typical run, calorimetric cells (sample and reference) are heated up with scan rates ranging between 0.5–1 down to 0.01 K/min. Several scan rates (temperature-time profiles) are usually investigated to optimise the proper equilibration time with the best signal-to-noise ratio, as low scanning rates produce small heat flow signals (energy per unit time). Distortion of the shape of the heat capacity function can be effectively corrected by the approximate Tian equation (Calvet and Prat, 1963). However, in view of the low scanning rates commonly used and the instrumental characteristic times (of the order of 100 s), this correction is taken as negligible under most experimental conditions.

Scanning microcalorimetry studies of helix ↔ coil transition of polysaccharides

Microbial polysaccharides are biotechnologically produced and have a paramount relevance in industrial food and non-food applications (Sutherland 1998). Their 'quality' resides in their reproducible chemical structure (contrary to many plant gums) and their ecological properties (contrary to many synthetic polymers). In addition to the valuable physical properties (they act as emulsion stabilisers, gelling agents, inhibitors of crystal formation, viscosity controllers), many of them exhibit biological properties which have been positively explored in biomedicine.



[1] a trisaccharide has been reported for the structural unit to represent an essentially linear polymer, while M_0 refers to the single monosccharide repeat unit

Fig. 5 Scheme of the structural architecture of polysaccharide repeat units. Molecular mass of the repeat unit is also indicated (numbers in parentheses refer to the units without non-sugar substituents)

The following sections report on the results of some DSC analyses of the cooperativity of the helix ↔ coil transition (Table 1), together with some relevant structural information of the polysaccharides studied. The list (see Fig. 4 for names and formulas) is not intended to be exhaustive, but only to cover a range of polysaccharides on which investigations have been accumulated, with some preference to microbial polysaccharides as biased by the authors' experience and of polymers importance. Figure 5 reports also the molecular masses of the repeat-

ing unit of the polymer in the 'idealised' native form, with a stoichiometric amount of non-sugar substituents as indicated. The molecular mass of the repeat unit, free from non-sugar substituents, is also shown since sample preparation may often include hydrolytic removal of these substituents.

Table 1 Thermodynamic data for the helix-coil transition of some polysaccharides

Polysaccharide	T_m/°C	ΔH/J g^{-1}	σ	N°
Xanthan (0.01 M NaCl)	45	12	$\approx 10^{-5}$	≈300
Schizophillan (in water)	≈135	≈27	$2.5 \cdot 10^{-5}$	200
Gellan	75	25	$\approx 10^{-2}$	≈8
CPS *Rhizobium* TA-1	47	22	$7 \cdot 10^{-5}$	120
Succinoglycan (0.1 M NaCl)	71	17	$4 \cdot 10^{-5}$	1 50
Agarose	40	18	$1.5 \cdot 10^{-4}$	80
Amylose (-iodine-triiodide)	50	57	$4 \cdot 10^{-2}$	≈5

Although the ordered structure in solution cannot be precisely determined, the assumption is usually made that it is essentially preserved from the helical form in the solid state. Therefore, a brief account of the helical parameters is given for each polymer (Rao *et al*. 1998).

Xanthan: X-ray data are not conclusive, although indicating that has a 5-fold helix symmetry and pitch of 4.7 nm (*c*-axis); none of the several models (single, double, parallel, antiparallel, left-, right-) provide acceptable X-ray fit.

Schizophyllan: A structure similar to that of hydrated curdlan is usually assumed, with c = 1.878 nm (h = 0.314) given by a 6-fold, parallel, right-hand, triple helix.

Gellan: Both native gellan and de-esterified (acetyl and glycerate) gellan (K^+ form) have been studied by X-ray, giving essentially a three-fold helix with c=2.815 nm (h = 0.913, 3-fold, left-handed, half staggered, parallel, double helix)

Succinoglycan: No data have been clearly published on X-ray fiber diffraction of succinoglycan. It has been quoted (Borsali *et al.* 1995) that it is a 'single helix' with a repeat length h = 1.92 nm, while most recent data (in solution) substantiate the existence of a double helix with a pitch of about 2 nm per repeat unit (Nakanishi and Norisuye, 2003).

CPS *Rhizobium* TA-1: The polysaccharide forms a 2-fold single helix of pitch 2.02 nm; since it is stabilised by a series of hydrogen bonds that involve the side chains, it has the appearance of a pseudo-double-helix.

Agarose: The original proposal is of a 3-fold, left-handed, half-staggered, parallel, double helix) with a pitch of c=0.95 nm (h = 0.633). Another set of data on dried films was interpreted as extended single helices with h ranging from 0.89 to 0.97 nm.

Amylose: Based on the energy contours several helical polymorphs are possible in view of the external conditions. The so-called hydrated 'V' amylose is characterised by a left-handed helical conformation with a pitch of 0.8 nm involving six residues per turn (h = 1.33 nm).

XANTHAN

Xanthan is a microbial polysaccharide produced by *Xanthomonas campestris*, the first bacterial polysaccharide to be food-approved by FDA in 1969 (and by EC in 1980). Its primary structure is constituted by a cellulose-like backbone of (1 → 4)–β-D-glucose residues with a trisaccharidic side chain composed by mannose, glucuronic acid and mannose, attached at C(3) and linked on alternate glucosyl residues. The proximal α-D-mannose residue is usually acetylated on C(6) while the distal β-D-mannose may present a pyruvic acid residue in ketal linkage at C(4) and C(6). The proportion of these substituents can be easily modified by mild chemical treatments (acidic or alkaline hydrolysis) or by changing strain and culture conditions (Sutherland 1998).

Although its peculiar thermally stable viscosity behaviour was immediately appreciated in many technological applications, its conformation in the native state was a matter of debate for a long time. Nowadays the most credited stable conformation in solution is that of a double stranded chain (Berth *et al.* 1996 and reference therein).

The thermally induced order-disorder transition of xanthan in aqueous salt solution has been detected by a number of physical methods, such as viscosity, optical rotation, differential scanning calorimetry. In particular, given the ionic character of the polysaccharide, the influence of the ionic strength on the transition temperature has been largely investigated in order to analyse its polyelectrolytic behaviour in the frame of polyelectrolytic theories. The reader is addressed to the basic theoretical background here not reported (Anderson and Record, 1990, 1995; Paoletti *et al.* 1985) and to its application to succinoglycan (Burova *et al.* 1996) and to other polysaccharides (Benegas *et al.* 1998). This type of analysis, corroborated by many independent measurements from different authors, has univocally assigned the conformational transition largely as a double-helix to coil.

A detailed statistical mechanical analysis was offered by Brant and coworkers to elucidate the thermodynamic aspects of the conformation and of helix stability of xanthan fractions subjected to thermal treatments (Hacche *et al.* 1987) up to a throughful exploration of its rheological properties (Lee and Brant 2002a, 2002b, 2002c). The transition has been seen as a partial melting of the double strand: from light scattering a decline in M_w as a function of temperature, on passing through T_m, was not seen even if expected and evidences showed that a significative amount of the dimers dissociate in water only at 95°C (Kawakami *et al.* 1991).

The possibility of analysing several results on xanthan homologous samples with different acetyl groups and/or different pyruvyl substituents opens an interesting opportunity to verify, within the accuracy of the experimental data,

some basic axioms of the helix-coil conformational transition of charged biopolymers. Among all these data, the enthalpy of melting ranges from ca 9 to 12 J/g (Christensen *et al.* 1993, Paoletti *et al.* 1983).

The contents of acetyl and pyruvyl residues strongly affect the polysaccharidic solution properties (Holzwarth 1979, Shatwell *et al.* 1990a, 1990b). These authors showed that acetyl groups have a stabilising effect on the ordered conformation and therefore increase the transition temperature while opposite effect can be attributed to pyruvate substituents; acetyl groups have the major effect on the shift in temperature. As for charged polysaccharides the transition temperature increases with increasing salt concentration and at constant salt concentration (below 1M) the transition temperature decreases with increasing pyruvate content (Kitamura *et al.* 1991).

Another series of xanthan derivatives has been prepared (Christensen, 1993) by depleting the terminal β-mannose residue in the side chains to a variable extent (f_M from 1.0 to 0), whereas the rest of the molecule remains essentially unchanged (however, also the acetyl group was always removed). The conformational transition of these samples, studied by optical rotation and calorimetry, has been analysed both in terms of the Zimm-Bragg theory and of the polyelectrolytic theory of conformational transition of charged polymers (Anderson and Record, 1990, 1995).

Values of the cooperativity parameter σ were evaluated from these data. Although the results are quite reproducible over different sample preparations, the scattering of the data as a function of f_M does not allow to extract a clear dependence (if any) on the content of the terminal mannose units. Taking for granted the self-consistency of calorimetric data alone, then σ should range between 10^{-4} and 10^{-5} with an upward parabolic curvature. The higher cooperativity for the unmodified sample and for the fully modified sample with respect to those partially modified is amply justified in terms of the perturbation of the ordered state, given by a statistical distribution of structural modifications.

The ionic strength dependence of the transition temperature would therefore return the non-ionic contribution to the transition enthalpy and the changes in the charge density of the polymer due to the conformational transition. Given the structural parameters that enter into equations, it is mandatory that the actual conformational states are known and that the transition occurs between two structurally defined states. The possibility of a time-dependent mixed population of double- and single-stranded makes difficult to properly analyse these data species, as previously suggested by Brant and co-workers. Under these circumstances, the unusual temperature dependence of light scattering data from fractionated xanthan samples was interpreted with the formation of both linear and cyclic structures, later confirmed by AFM investigations (McIntire and Brant, 1997).

SCHIZOPHYLLAN

Non-ionic glucans with a ß-1,3 sequence of glucose are produced by many microorganisms and include the curdlan family and the scleroglucan-schizophyllan

family. Schizophyllan primary structure consists of linearly linked ß-1,3-D-glucose residues with one ß-1,6-D-glucose side chain every third main-chain residues. In water and at room temperature the polysaccharide exists as triple helices (Norisuye, 1980; McIntyre and Brant, 1998) made up by three chains interacting by intermolecular hydrogen bonds with the side arms outward of the helix. This ordered conformation is still preserved even in presence of DMSO up to 70% (Kitamura and Kuge, 1989) while a triple-helix to single-coil transition occurs at higher DMSO contents (Sato *et al.* 1983). Conformational transitions can be also thermally induced, in various solvent compositions; the polymer presents a highly cooperative order-disorder transition in the side-chain conformation at low temperature and a dissociation-disordering transition at high temperature.

Regarding the low temperature transition, in water schizophyllan exhibits considerable changes in optical rotation (OR) and heat capacity at about 6°C (Itou *et al.* 1987). The small endothermic peak appears also in the experiments made by Bot *et al.* (2001), as well as in those reported by Yoshiba *et al.* (2002). Itou *et al.* (1986) proposed a long distance organisation of the side chain in which water molecules plays an important role, organisation that evolves toward a disordered form by increasing temperature. The fact that this conformational transition at low temperature is affected by the substitution of water molecule with D_2O, supports this hypothesis (Itou *et al.*, 1987).

In a detailed calorimetric study (Kitamura and Kuge, 1989), high-sensitivity DSC was shown to be a very useful tool for investigating thermally induced conformational transitions of this polysaccharide. The original paper reports a set of DSC curves depicting the phase diagram for the conformational transition of a low molecular weight ($Mw = 1.34\ 10^5$) schizophyllan in water-DMSO mixtures. Values of ΔH_{cal} are reported for the two transitions at several solvent compositions. For the triple-helix to coil transition a value of 27 J/g (in water at $T \approx 135$°C) can be extrapolated from experimental data as a function of T and solvent composition. The ratio of the van't Hoff to calorimetric enthalpy, related to the size of the cooperative unit, raises from ca 70 up to 200 with increasing water concentration. In the context of this study, Kitamura and Kuge (1989) neatly showed how to reconcile the previous literature results, which seemed inconsistent only because they were incomplete. Not only does the complete phase diagram of schizophyllan in water-DMSO clarify such a discrepancy, but moreover, the direct calorimetric determination of transition enthalpy has provided further insight to the energetics and cooperativity of the two conformational processes.

In a more recent work (Kitamura *et al.* 1996) it is proved that ΔH_{cal} is independent on the pH of the solution and, at a constant pH, is also independent on added salt. The ratio $\Delta H_{vh} / \Delta H_{cal}$ leads to a cooperative units size of about 300 for pH below 10 that decrease to a value of approximately 30 with increasing pH. Although an analogous diminishing in the cooperative unit size was observed due to addition of DMSO, the cooperative length is less sensitive to the addition of DMSO and the given explanation concerns the preferential solvation of the polymer by

DMSO. The solvent effect on the cooperativity has also been shown by Hirao *et al.* (1990); as the DMSO content increases in a schizophyllan/D_2O solution, the transition shifts towards higher temperature but the cooperativity in terms of σ is almost the same for the different solvent compositions under study. The transition at higher temperatures is characterised by an asymmetry of the DSC curves which, however, could be accounted for on the basis of a simultaneous conformational transition and dissociation process.

GELLAN

Gellan is a bacterial polysaccharide produced by the micro-organism *Sphingomonas elodea* with a primary structure consisting of a regular sequence of tetrasaccharide repeat units in the backbone composed of glucose, glucuronic acid and rhamnose at a molar ratio of 2:1:1. The native polysaccharide contains an acetyl and an L-glyceryl as substituent on one of the glucose unit and forms a soft and elastic gel. Deacylation by alkaline treatment gives gellan in its commercial form. The commercial polymer is able to form rigid and clear gels which are in some respects comparable with those formed by agarose. X-ray diffraction studies have shown that in the solid state gellan exists as an extended intertwined, three-fold left handed double helix (Chandrasekaran *et al.* 1995). The glyceryl group enhances the stability of the double helix, whereas the acetyl group does not interfere with packing arrangement and hence has no structural influence.

Identification of ordered conformations of gellan in solution is complicated by its ability to aggregate and form intermolecular ordered structure. However, several facts argue in favour of the double helix conformation in dilute solution at low temperatures; from small-angle X-ray scattering of commercial gellan in aqueous solution, the relative linear mass density (polymer concentration 1.0–1.5%) at 10°C was reported twice that at 60°C (Yuguchi *et al*. 1996). The increase of polymer concentration (2.9 and 5.7%) led to larger values confirming the further association of double helices. This double helix association, essential for gel formation, is controlled by the type of counter ions. The cations role (for deacylated gellan in sodium salt form in the presence of calcium and potassium ions) has been recently studied by transmission electron microscopy (Atkin *et al.* 2000). The cation type and the cation:carboxylate concentration ratio (below, above or at the stoichiometric equivalence) have a profound influence on polymer morphology with evidence of lateral aggregation of the thermodynamically stable conformation of gellan in salt-free aqueous solution (double helix and double-helical duplexes).

Differential scanning calorimetry studies of 10 mg/mL gellan in the absence of added salt showed single thermal transition on heating and cooling that has been attributed to coil-helix transitions. At polymer concentrations higher than 32 mg/mL DSC heating curves show two endothermic peaks; the lower temperature transition was attributed to aggregate-helix melting and the high temperature transition to helix-coil melting (Miyoshi, *et al.* 1995a, b). Similar

studies (Mazen *et al.* 1999) showed that at fixed polymer concentration (10 mg/mL) and low ionic strength (0.01 M NaCl) thermograms of deacylated gellan are characterised by a single peak on heating and cooling at relatively low temperature (ca. 34°C) with a very small hysteresis. The enthalpy of the process, attributed to the helix-coil transition, was reported to be 9.5 J/g. On increasing the salt concentration to 0.05 M a second peak at higher temperature appeared on heating (42°C) and at 0.1 M two well separated peaks were present in the thermograms. The first peak (for 0.1 M NaCl at ca 53°C), attributed to the helix coil transition appeared nearly located at the same temperature as the peak on cooling runs. The second DSC peak (for 0.1 M NaCl at ca. 75°C) was related to the formation of large aggregates of double helices. The enthalpy of the conformational transition increases progressively with the salt concentration (up to 18 J/g) as expected for polyelectrolytes.

In order to avoid contributions related to secondary aggregation process, deacylated gellan in tetramethylammonium (TMA) salt form (salt concentration range: 0.0025–0.5 M TMACl) in dilute solution (polymer concentration: 0.5–0.7 mg/mL) has been recently studied by means of high sensitivity DSC (Grinberg *et al.* 2003). Some common features of the transition have been observed at every salt concentration like, for instance, the presence of a single heat absorption (with position, size and shape depending on ionic strength) and the λ-like profile (long tail to the left of the maximum and an apparent break point to the right of it) with a very sharp maximum of the thermograms. Similar profiles were observed for a highly purified sample of sodium salt gellan (10–15 mg/mL) in salt free solution (Miyoshi, *et al.* 1999). Moreover, no distortion of the transition λ-like profile was observed in the whole range of ionic strength confirming that the double helices of the TMA gellan are not capable of aggregation. Both transition temperature and enthalpy increased with increasing salt concentration as expected for charged linear biopolymers. In particular, the enthalpy of the process has been reported to be confined in the range 5–10 kJ/mol within the investigated ionic strength range. By analysing the profile of the conformational transition with a model which considers two sources of cooperativity of the double-helix transition (stacking and loop factors) the authors led to the conclusion that the cooperative unit of gellan involves about eight repeating units (close to the persistence length of the disordered gellan chain) with a cooperativity parameter (0.62±0.01) indicating that the partial unfolding of the double helix in its middle section (loop effect) dominates the cooperativity of gellan transition. Moreover, in order to fit the Poisson-Boltzman model to the experimental free energy of transition against the concentration of the salt at $T = 273$ K it is necessary to suggest that the effective linear charge density of gellan in the coil conformation is larger that that estimated for the fully extended chain.

Native gellan (0.8 acetyl and 0.8 glyceryl substituents per repeat unit) conformational transition has been also investigated by DSC (Mazen *et al.*

1999). Calorimetric data confirm that the native gellan is a double helix with a much higher thermal stability than the deacylated one due to the role of the glycerate groups (deacetylation have nearly no influence on the stability of the double helix). In facts both transition temperature and enthalpy have been reported significantly higher than those of commercial sample. The enthalpy ranges from 18 to 22 J/g (polymer concentration 10 mg/mL), passing from 0.01 to 0.1 M NaCl. Contrary to deacylated polymer, the ionic strength dependence of transition temperature of native gellan is relatively small and on increasing salt concentration, only one peak on thermograms has been observed. The role played by glyceryl groups, by perturbing the conformational transition, has a correspondence in the modification of rheological properties and of the packing density in the solid state.

CAPSULAR POLYSACCHARIDE FROM RHIZOBIUM TRIFOLII TA-1

The chemical structure of *Rhizobium trifolii* capsular polysaccharide (TA-1-CPS) is characterized by a trisaccharide in the chain backbone which possesses two branches on the same glucosidic residue. The most dramatic solution property exhibited by TA-1-CPS is, by far, its ability to form aqueous thermo-reversible gels in a wide range of polymer concentration (down to ≈ 0.1 g/L). In particular, due to the non-ionic character of the polysaccharidic chain, gels can be formed in the absence of ionic co-solutes and show a remarkable gel strength. A number of experimental observations (Cesàro *et al.*, 1987; Gidley *et al.*, 1987), in particular on the thermal and rheological behaviour of TA-1-CPS in the presence of co-solutes (urea, salt, or sucrose), suggest that at least three different levels of structure may be involved in the process of aqueous gel formation. While the first level was referred to as local chain conformational ordering, it was thought that the second one involved 'intermolecular ordering between conformationally ordered segments'. This structure has been shown to resist shear and such denaturants as urea. The third level of structure provides for the three-dimensional gel network and is labile under moderate shear and in concentrated urea solution: it involves supramolecular aggregation. Evidence for a complex aggregation in the development of the gel structure has also been accumulated from independent experimental work (Cesàro *et al.*, 1987 and Gidley *et al.*, 1987). In particular, both the hysteresis and the temperature dependence of the rigidity (storage) modulus in water and in aqueous urea solution support the presence of an intermediate step for the formation of an aggregate structure. From the structural point of view, although the quality of the diffraction pattern of the TA-1-CPS did not at first permit a good resolution of its structure, the layer line spacings show that the chain has a 2-fold helical symmetry with a chain repeat axis of 0.98 nm per repeating unit (Lee *et al.* 1992).

For this polysaccharide, calorimetric experiments were carried out under similar conditions and with three different high-sensitivity DSC instruments. The polysaccharide was repeatedly heated and cooled, and the thermal curves

were almost completely reproducible. The DSC results showed a very sharp transition at around 47°C. The transition, indeed, was reversible, sharp but asymmetrical (related to the aggregation). From the direct calorimetric heat of transition of 22.2±0.2 J/g and the van't Hoff enthalpy of about 1255 kJ/mol, which was estimated according to the procedure outlined above, the molecular weight of the cooperative unit $N°$ resulted as 57000. This value brings the length of the cooperative segments to about 57 nm which stabilises the ordered helical conformation in the gel structure.

Therefore, gelling properties of CPS arise from a stabilised array of energetically favourable overlaps between the side chains, while the stereoregular non-ionic main-chain maintains a helical conformation in water, which, most probably is the same as that found for CPS fibres by means of the new X-ray diffraction data (Chandrasekaran *et al.* 1992). It is interesting to note that the presence of side chains on a polysaccharide backbone is normally considered a perturbing factor with respect to gelation. Examples can be taken from literature and include the welan-rhamsan family as well as the curdlan-schizophyllan case. The case of TA-1-CPS, however, points to the opposite. In fact, either the cleavage or modification of the side chain destroys the ability of this polysaccharide to form a gel. A scrutiny of the results concerning the conformational transition induced by temperature on derivatives, obtained by sidechain partial modification, led to the conclusion that the gel stability decreases linearly with the side chain modification, which must destroy the functionality of the arms in the intermolecular cross-linking process (Cesàro *et al.*, 1989).

Let us quote here that the same strain of *Rhizobium trifolii* produces an abundant quantity of exocellular ionic polysaccharides (TA-1-EPS) which also exhibits a ionic strength dependent conformational transition (Crescenzi *et al*. 1987a, 1987b). In addition, other microrganisms offers polysaccharides with the same primary structure but naturally differing in the amount of non-sugar substituents (Faleschini 1988; Cosani *et al*. 1989; Cesàro *et al.* 1992). Although this would have offered an interesting case of homologous sample, detailed analysis of calorimetric data has not been published.

SUCCINOGLYCAN

Succinoglycan is a microbial exopolysaccharide produced by several strains of soil bacteria belonging to the genera *Alcaligenes*, *Pseudomonas*, *Agrobacterium* and *Rhizobium*. The polymer chains are made up of octasaccharide repeat units. Four monosaccharides of every repeat unit (three *D*-glucose and one *D*-galactose residue) make up the backbone, where the galactosylated glucose residue serves as a branching point bearing the tetrasaccharide side chain composed of *D*-glucose residues at position 6. Two charged non-carbohydrate substituents (succinate half-ester and 1'-carboxyethylidene acetal) are located in this side chain, whereas O-acetyl groups, when present, may be found in the backbone. Succinoglycan does not form gels, but give rise to extremely viscous solutions

or weak gels (Cesàro *et al.* 1992), however at sufficiently high polymer concentration and in dependence of sample origin and thermal history of its aqueous solutions substantial aggregation can occur leading eventually to the formation of thermoreversible gel (Boutebba *et al.* 1999).

According to most recent light scattering and viscometric data (Kaneda *et al.* 2002; Nakanishi and Norisuye 2003), succinoglycan in salt solution (0.01 and 0.1 M NaCl at 25°C) behaves as a rod-like polymer with a persistence length of 50–180 nm and a molar mass per contour length of 1510 nm^{-1} (corresponding to that of a double-helix). At 75°C, the polymer behaves as a worm-like chain with a persistence length and a molar mass per contour lenght of 10 nm and 750 nm^{-1} respectively. From these data, the polysaccharide is considered to be a dimer that has ordered structure of double helical nature. However, in salt-free solution, it was suggested that succinoglycan behaves as a single chain in relatively low polymer concentration (Borsali *et al*. 1995).

Up to now, the thermally induced order-disorder conformational transition of succinoglycan has been studied by high sensitivity calorimetry only in one detailed study, even if scarce calorimetric data have frequently been included in studies aimed at characterizing the polymer solution properties. The complex nature of the succinoglycan order-disorder conformational transition has been studied (Burova *et al.* 1996) by examining the concentration dependence of the transition temperatures and the shape of the excess heat capacity curves obtained by high-sensitivity adiabatic DSC (5–100°C, heating rate 1 K min^{-1}). Thermograms of succinoglycan in salt-free solution at polysaccharide concentrations of less than ca. 2 mg/mL, have been satisfactorily described by the two state model suggesting the transition mechanism to be of the single helix-coil type. At higher polymer concentration, the transition curves become characterised by a marked asymmetry and are described by a polysteric model which includes two stages: the cooperative dissociation of the helix dimer and subsequent melting of helix monomer. At NaCl concentrations 0.01 and 0.1 M thermograms have been well fitted by the polysteric model within the whole studied range of polymer concentration (0.1–3.5 mg/mL).

The theoretical profile of the transition was therefore calculated by using an application of the general allosteric approach developed by Gill's group (Robert *et al*., 1989) According to this model, based on the theory of the helix ↔ coil transition, the fitting approach gives the number $N°$ of octasaccharides in the cooperative unit of succinoglycan. Furthermore, the results suggest that the average length of a succinoglycan helix increases with salt concentration but does not depend on polysaccharide concentration. The average value of $N°$ changes from 85±25 in water to 150±20 in NaCl 0.1 M, in agreement with the indications of the scattering data. As an example, a chain of succinoglycan with molecular weight $5.4\ 10^6$ in aqueous 0.1 M NaCl solution includes more than 10 «independent» helix segments each made up of an average of 150 repeat units. Therefore, the stability of such a system, consisting of a sufficiently large number of these extended

segments, should be fairly insensitive to moderate variations in the chain-length. For this reason, the effect of the molecular weight heterogeneity of the sample on the profile and parameters of the conformation transition for succinoglycan appeared to be negligible according to the authors (Burova *et al.*, 1996).

The total value for the enthalpy of transition of succinoglycan double helix to single coil chain (average upper limit of 17.34 J/g) is given by the sum of the double helix dissociation enthalpy and the melting enthalpy. The contribution of the dissociation enthalpy of 6.67±0.6 J/g has been calculated. The contribution of melting enthalpy is strongly influenced by polymer concentration in salt-free solution (from 5.34 J/g at 0.1 mg/mL to 10.67 J/g at about 2.0 mg/mL with a S-shape profile). In 0.01 M NaCl the melting enthalpy pass from 8.00 J/g at 0.1 mg/mL to 10.67 J/g at 2.5 mg/mL. A constant value of 10.67 J/g in the whole range of polymer concentration is observed in 0.1 M NaCl. The peculiar behaviour emerging from this calorimetric study, not only permits to interpret some previously unclear issues but also to reconcile some discrepancies of previous studies. For instance, the transition enthalpy reported by Ridout *et al*. for native succinoglycan, 9.47 J/g, the value reported by Fidanza *et al*. 14.40 J/g and the values reported by Boutebba *et al.* 17.1–18.8 J/g are presumably obtained under different experimental conditions.

The cooperativity parameter has been reported to range from $14\pm6\ 10^{-5}$ (salt-free solution) to $4.4\pm1.2\ 10^{-5}$ (0.1 M NaCl) indicating that the melting process of succinoglycan helices is a highly cooperative transition in comparison with other biopolymers (Burova *et al*. 1996). The presence of non-carbohydrate substituents has stimulated investigations on the role played by O-acyl and pyruvyl residues on the stability of the succinoglycan ordered conformation. Preliminary DSC studies on acetyl-containing succinoglycan sample in salt-free aqueous solution (Ridout *et al*. 1997) revealed that removal of the acetyl substituents does not improve the cooperativity of the transition and reduce the stability of the helix whereas removal of succinyl groups raises the thermal stability of the helix, increases the transition enthalpy and improves the cooperativity of the transition. The latter behaviour has been suggested explainable in terms of the reduction in charge density on the polysaccharide chain.

ALGAL GALACTANS: AGAROSE

Agarose is a neutral algal polysaccharide, ideally constituted by alternating residues of 1,4-linked 3,6-anhydro-α-L-galactopyranose and 1,3-linked β-D-galactopyranose. The polysaccharide is the neutral member of the agar family extracted from red algae and is usually associated with the sulphated galactans (carrageenans), which however, in addition to the charged sulphate groups, present a different sugar stereochemistry. The widespread empirical use of agarose in the preparation of neutral gelled substrate for the electrophoretic separation of valuable biological material has often obscured the efforts made to elucidate the gel microstructure and its molecular architecture (Maaloum *et al.*, 1998). The gel is

stable at room temperature and is characterised by a high rigidity. The hysteresis between the melting temperature (in the range of 85–95°C) and the gelling temperature (25–35°C) is rather pronounced, although recovery of the gel properties usually occurs after heating and cooling cycles. Furthermore, while the specific enthalpy of melting does not change with polysaccharide concentration, the gelling temperature increases asymptotically with concentration, with a limiting upper temperature of about 40°C. Unfortunately, despite the different temperature range for the melting and gelling phenomena, no clear cut analysis has been made for the two distinct processes, and the tacit underlying assumption seems to be that the system is effectively in a sort of 'delayed' equilibrium. Comparative data for the agarose gelling process can be taken from the temperature dependence of the dichroic absorption as reported by Fujii *et al.*, (2000) and from the calorimetric DSC data reported by Rochas (1987). When the calorimetric enthalpy is set at 18.28 J/g (5.6 kJ/mol of repeat units), the van't Hoff enthalpy obtained from the CD data is 450 kJ/mol, giving a value of about 80 units for the cooperative length. It is important to stress that this analysis cannot provide any information as to whether the helix-coil transition involves single or double helices (either with intertwined or side-by side geometry); this information can only be obtained from direct structural analysis. The value of 80 units relates only to the meaning of the size of the cooperative block which melts simultaneously, no matter whether it is a single linear chain of 80 units or 10 associated chains each of 8 units. It is, therefore, rather surprising that the divergence between the calorimetric enthalpy and the van't Hoff enthalpy has been taken, at different times by both these authors, as evidence for the contribution of the helix-helix interaction among agarose fibres, which is claimed to be considerably larger than the conformational contribution in the coil-helix transition. Needless to say, also the size of the molecular unit in the equilibrium constant has been misinterpreted, taking into account a hypothetical macromolecular weight of 120 000 for the agarose polysaccharide. Given the context of a scaling analysis of rheological properties of agarose gel, the molecular dimension of the ordered blocks may also play a role in the 'chicken wire' network responsible for the elastic properties. It has also to be taken into account that most recent calorimetric data give a 'moisture' dependent heat of transition, providing a limiting value at high water content of 57.6 J/g (Cooke *et al.* 1996). Whether the data above reported could be re-evaluated in the frame of a suitable model of helix-coil transition, is matter of future debate.

STARCH AMYLOSE

The essentially linear $\alpha(1–4)$ glucose polymer is named amylose; together with the highly branched amylopectin constitutes the polysaccharidic component of starch. By specifically referring to amylose molecule (i.e., in the absence of amylopectin) we wish to avoid the confusion of attributing to starch as a whole the properties of individual components, a problem that seems quite common in