

# Polymerization in Biological Systems

Ciba Foundation Symposium 7 (new series)



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# Polymerization in Biological Systems

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# Introduction: the objectives

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At this symposium we have brought together polymer chemists, who are mainly interested in polymerization and the properties of synthetic polymers, and biologists, who are mainly interested in the biosynthesis, structure and biological function of biopolymers. Biologists realize by now that a thorough understanding of the synthesis and behaviour of biopolymers both *in vivo* and *in vitro* requires a correspondingly thorough knowledge of chemistry and physics. At the same time chemists have been developing new theoretical and experimental techniques which offer new lines of approach to those biologists who are primarily concerned with the formation of biopolymers and their modes of intra- and intermolecular interaction. I hope, therefore, that at this symposium we shall find a common language to cope with the problems to be discussed.

The layman often refers to this age as the age of plastics, in which nylon, polystyrene, polyvinylchloride and other synthetic polymers are in everyday use. Biopolymers, such as proteins, nucleic acids and polysaccharides, play a major role in determining the most essential processes in living organisms. They have, in one form or another, been utilized since time immemorial, and by now even the man in the street is familiar with some of their specific biological properties, yet the interests of the molecular biologists are still rather different from those of the synthetic polymer chemist. The polymer chemist is mainly interested in the mechanical, electrical and other physical properties of the polymers which he has produced. The molecular biologist is interested in nucleic acids, proteins and other biopolymers because they store information, because some of them act as catalysts, and others possess recognition patterns which allow them to recognize other molecules. There is much room for increased cooperation between the two groups of scientists with their somewhat divergent interests.

We are going to begin by discussing polymerization mechanisms and the techniques used in the preparation of synthetic polymers. In some cases, the mechanisms involved in the formation of synthetic polymers resemble those observed in the biosynthesis of natural polymers, e.g. condensation. However, the commonly used polymerizations, in which radicals and carbonium ions participate as reactive intermediates, have no counterpart in nature. I wonder why this is so?

Professor Fritz Lipmann, who most certainly is not a synthetic polymer chemist, has divided the reactions by which biopolymers are formed into two classes: the tail type of polymerization and the head type of polymerization (Lipmann 1968). The tail-type polymerizations are those in which the polymer has an inert tail with which activated monomers react successively. In the head type of polymerization the head of the polymeric chain is active; elongation of the polymeric chain occurs as a result of the reaction of the active head with the monomer generating a fresh active head. Why does nature choose the tail type of polymerization to produce polysaccharides, RNA and DNA, and why is the head type of polymerization used in the synthesis of proteins, lipids and terpenes? Certainly nature has had a long time to try out various types of polymerization before making its choice.

The molecular biologist studying the biosynthesis of natural macromolecules has gradually come to realize the complexity of the reactions involved. The classical biochemist is mainly familiar with homogeneous enzymic reactions. In fact he usually does his best to study enzymic reaction in dilute aqueous buffer solutions. Unfortunately, many of the biologically important polymerizations occur in heterogeneous media. Protein biosynthesis, for example, requires the participation of ribosomes, ribosome aggregates, the polysomes and numerous protein factors; moreover the endoplasmic reticulum seems to play a major role in organizing and regulating the process. This is somewhat annoying for the classical biochemist; it is also somewhat daunting for the classical synthetic polymer chemist who is aware of the fact that even 'simple' polymerizations catalysed by heterogeneous catalysts and leading to isotactic and syndiotactic polymers are still far from being thoroughly understood. Even the relatively simple biopolymerizations involved in cell wall formation present many intricate problems still to be solved.

In spite of the complexity of biopolymerizations, the molecular biologists have succeeded in recent years in reproducing *in vitro*, partially or completely, many such processes. It is thus possible to study in the test tube the various factors involved in the biosynthesis of proteins, polysaccharides and nucleic acids. The replication of nucleic acids *in vitro* has, moreover, allowed the study of evolution at the molecular level. The theoretical implications for molecular

evolution of the beautiful experiments of Dr Spiegelman with Q $\beta$ -replicase will be discussed by Dr Eigen.

During the symposium we shall spend some time discussing the factors determining the secondary structure of synthetic polymers and biopolymers. Starting in the late 1940s, the polymer chemists attempted to derive many of the properties of synthetic linear polymers, both in the solid state and in solution, from a statistical treatment of the random coil (see, for example, Flory 1953). In recent years, however, it has become evident that intramolecular atomic interactions, bond lengths, bond angles and bond rotational potentials severely restrict the conformations which a linear polymer chain can assume. Furthermore, polymer-solvent interactions play an important role in determining the conformation of polymers in solution. In coping with these problems the polymer chemist has devised new theoretical approaches for predicting the macromolecular conformations of lowest free energy (Flory 1969). The new theoretical tools thus developed have been adopted by the theoretical protein, polysaccharide and nucleic acid chemists for evaluating some of the factors determining the highly specific conformations assumed by native biopolymers.

The above theoretical approach is essentially a thermodynamic one; the biologist, however, is interested not only in the relatively stable macromolecular conformations but also in the mechanisms and reaction rates involved in the transformation of a given polymer from one conformation to another. The biologist wants to know how fast a polypeptide chain coils into its active three-dimensional structure, how fast a double helix forms, and how fast it unwinds. Furthermore, rather subtle conformational changes seem to be directly involved in the activity of enzymes, antibodies and receptors, as illustrated by the recent findings of Perutz (1970) on haemoglobin and Lipscomb *et al.* (1969) on carboxypeptidase. Information as to the rates of such conformational transitions is thus crucial to the understanding of specific biological interactions. Unfortunately kinetic data on the rates of conformational transitions of both synthetic and biopolymers are still scanty.

In current statistical mechanical analyses of chain molecules it is still difficult to take into consideration interactions between residues far apart on the linear polymer chain. Such interactions are, however, of great importance in the determination of the tertiary and quaternary structures of proteins and other biopolymers.

Nature possesses a variety of means of attaining the appropriate conformations, associations and aggregations of biopolymers. These may be the direct result of the primary structure of the biopolymer. They may also result from the chemical modification or cross-linking of suitable residues, or from the

reversible binding of metal ions, cofactors, or other ligands. In some cases polymers possessing a higher molecular weight than the final product are formed initially and are subsequently enzymically degraded to yield the final species. Phosphorylation of enzymes by the appropriate kinases can serve as an example of conformation and biological activity being modified as a result of chemical modification (for review see Holzer & Duntze 1971). Disulphide bridges in fibrillar and globular proteins are known to stabilize their secondary, tertiary and quaternary structures markedly. The transformation of zymogens to active enzymes as a result of partial proteolytic degradation has been shown in some cases to lead to a marked conformational change. Similar changes most likely occur when proinsulin is transformed into insulin and fibrinogen into fibrin. It has been shown recently that even formation of the triple helix of collagen is preceded by the formation of a procollagen molecule possessing a considerably higher molecular weight than collagen (Bellamy & Bernstein 1971). The two ribosomal RNA fractions also stem from a single polynucleotide chain which is cleaved enzymically to yield the desired fragments (Grierson *et al.* 1970).

In the latter stages of the symposium we shall concentrate on assembly processes, i.e. on interactions between the subunits of a given protein, and on protein-protein, protein-nucleic acid and protein-lipid interactions. All these specific interactions between macromolecules depend largely on the ability of one molecule to recognize another. This feature, which is so characteristic of biopolymers, has not been utilized by synthetic polymer chemists but it strikes me as being one of the most important characteristics of the living organism. This is how hormones recognize receptors, how an antibody recognizes an antigen, and maybe how one cell recognizes another. In this connection it is worth mentioning, for the sake of our polymer chemists, that the recently developed technique of affinity chromatography is also based on the ability of a synthetic macromolecule, suitably modified, to recognize a corresponding biopolymer (Porath 1967; Cuatrecasas & Anfinsen 1971). The affinity chromatography technique is currently being extended to the isolation of cell receptors, and even to the separation of specific classes of cells.

We shall obviously not be able to cover thoroughly all the topics I have outlined. I hope, however, that those who discuss a particular topic will state clearly what has been achieved, what are the focal problems to be solved, and what theoretical and experimental difficulties may be encountered in so doing.

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# Synthetic polymers, biopolymers and block polymers

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*Abstract* During the last two decades two important factors have become controllable in polyaddition, the technique yielding synthetic polymers of high molecular weight. Coordination or ionic propagation provided means to direct in a highly specific way the stereochemical course of the reaction, and the utilization of living polymers allowed us to regulate the molecular weight of the resulting polymers and to produce them in uniform size. Moreover, the use of living polymers permits the preparation of regular block polymers—long-chain molecules composed of sequences of monomers of desired length which follow each other according to a predetermined pattern.

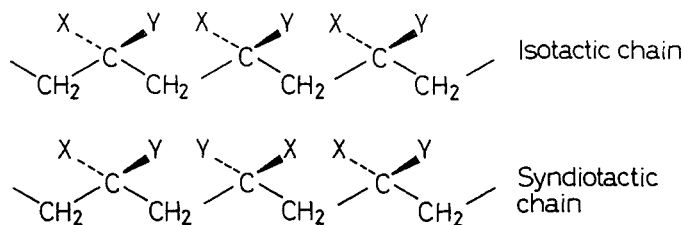
Examples of stereoregular polymerization will be reviewed and the factors responsible for the stereospecificity discussed. The cationic polymerization of some anhydro sugars, developed by one of us, will be considered in greater detail. This reaction yielded, for the first time, synthetic linear polysaccharides of uniform structure, such as dextran, which exhibit optical and biological activity.

The incompatibility of polymers built from different units leads to the formation of mesomorphic structures in block-polymer systems. The principles governing the mesomorphic structures will be discussed and examples will be given of new types of materials obtained by this route.

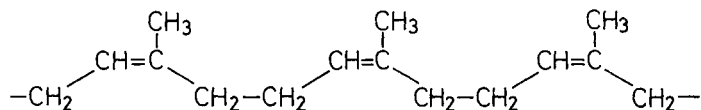
Two distinct techniques, polycondensation and polyaddition, are used in the synthesis of high molecular weight polymers, but only the latter is discussed in this review. Three basic steps are involved in a typical polyaddition: the active centres from which polymeric molecules evolve are produced in the initiation step, monomeric units are added consecutively to growing polymers during propagation, and finally the ability to grow possessed by each active end of a growing polymer is eventually lost in some termination step. The propagation steps determine the stereochemical structure of the resulting polymer and the termination steps govern their molecular weight and molecular-weight distribution.

## STEREOSPECIFIC POLYMERIZATION

In the past, synthesis of polymers led to random structures, that is—to apply modern terminology—the resulting product was atactic. Similarly, copolymerization of two or more monomers led to their random distribution along the polymer chain, alternating copolymers being an exception. The advent of ionic and coordination techniques of polymerization radically changed the situation. Natta made the striking discovery (Natta 1955; Natta *et al.* 1955) that stereospecific polymerization is both feasible and profitable on an industrial scale. His preparation of isotactic polypropylene was followed by numerous syntheses of other isotactic and syndiotactic vinyl or vinylidene polymers, that is polymers with identical or alternating pseudo-asymmetrical carbon atoms along their chains:



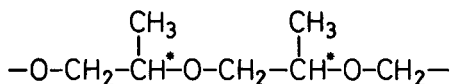
The anionic polymerization of isoprene performed in hydrocarbon medium with  $\text{Li}^+$  acting as the cation led to all *cis*-1,4-polyisoprene,



a synthetic material closely resembling the natural rubber. Soon after, catalysts were discovered which allow us to synthesize all *cis*-1,4 or all *trans*-1,4 polydienes, e.g. the synthetic all *trans*-1,4 polyisoprene represents a man-made analogue of another natural product—guttapercha. The power of stereoregular polymerization is more appreciated if one recalls that four distinct stereospecific polymers, namely, all *cis*-1,4-polybutadiene, all *trans*-1,4-polybutadiene, isotactic all 1,2-polybutadiene and syndiotactic all 1,2-polybutadiene, were produced from the same monomer—butadiene.

The techniques of stereospecific polymerization have been extended to other classes of monomers, for example to cyclic ethers. Price & Spector (1965), using ion-complex catalysts, synthesized a stereoregular polypropylene oxide from optically active propylene oxide although racemization occurs when the

polymerization is performed under conventional conditions. This polymer possesses truly asymmetrical carbon atoms in its backbone:



that is, the product is optically active if produced with the aid of a proper ionic (or coordination) catalyst from pure enantiomorph. This observation leads to a question: can a stereospecific system be devised which polymerizes only one enantiomorph in a racemic mixture? This problem has been examined by Pino (1965), who designed stereoselective catalysts and discussed their applications. Further development of stereospecific polymerization led to the synthesis of polymers in which each pair of two consecutive carbon atoms has a specific structure; thus, all *threo* or *erythro* chains have been prepared.

It is not possible to discuss in this brief review all the details of stereospecific polymerization. Suffice to say that the stereoregular polymers often show properties much different from those of the atactic (random) polymers and, hence, they have considerable practical as well as academic value. It would be advantageous, however, to consider the reasons why the stereospecificity operates in ionic or coordination polymerization and not in a radical polymerization.

The addition of a radical to a C=C bond is not guided by any preferential direction, although steric hindrance, usually not a very compelling reason, may favour one mode of the addition rather than another. In ionic or coordination addition a specific direction is introduced by the cation-anion axis of an ion-pair or coordination complex. This differentiates between the various modes of the addition and favours a stereospecific behaviour of the system. We therefore do not expect to find high stereospecificity in a process involving free ions.

A second and even more important factor favouring stereospecificity of ionic or coordination polymerization arises from the monomer-counter-ion interaction. The monomer, as well as the penultimate units of the growing polymer, may solvate the counter-ion and form an organized complex. The structure of such a complex compels the subsequent addition of the monomer to the growing polymer to proceed in a well-defined fashion. Moreover, the simultaneous coordination of the penultimate unit and the monomer with the counter-ion 'informs' the monomer about the configuration of the growing chain. Indeed, Fowells *et al.* (1967) demonstrated that the addition of selectively deuterated

acrylate or methacrylate,  $\begin{array}{c} \text{H} \quad \text{X} \\ \diagdown \quad / \\ \text{C} = \text{C} \\ / \quad \diagdown \\ \text{D} \quad \text{Y} \end{array}$ , leads to an orderly array of the  $\begin{array}{c} \text{H} \quad \text{D} \\ \diagdown \quad / \\ \text{C} \\ / \quad \diagdown \end{array}$  groups in the resulting isotactic polymer when the monomer, before

its addition, acts as a solvating agent of the counter-ion, i.e. when the polymerization takes place in a poorly solvating medium the molecules of which do not compete efficiently with the monomer for the solvating sites. However, in a better solvating medium this preferential orientation is lost because then only solvent molecules participate in the solvation of the counter-ion.

The mode of coordination of the monomer to the counter-ion is even more specific in coordination polymerization in which the monomer becomes a true ligand before its addition to the chain. In such systems the nature of other ligands is of paramount importance in determining the stereospecificity of polymerization and, indeed, the stereospecificity may be altered by replacing one set of ligands by another.

### LIVING POLYMERS

The anionic mode of polymerization allows us to control another feature of the reaction, namely its termination step. Termination is virtually unavoidable in radical polymerization because two radicals combine or disproportionate on encountering each other and either reaction leads to their annihilation, and hence to the loss of their reactivity. Such a bimolecular termination does not take place in ionic polymerization and, in fact, in proper conditions termination may be entirely eliminated in anionic polymerization (Szwarc 1956). The resulting polymer is known as living polymer; it ceases to grow when all the monomer is polymerized\* but it retains its ability to continue its growth whenever additional monomer is supplied.

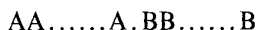
The concept of living polymers has numerous ramifications. Since polymerization proceeds without termination it becomes feasible to prepare polymers of nearly uniform molecular weight (having a Poisson distribution of molecular weights). Indeed, such polymers are produced now on a semi-industrial scale. Although spontaneous termination is eliminated a deliberate conversion of active ends of living polymers into inactive ends is still feasible. For example, the carbanions of living polystyrene or polydienes may be deprived of their ability to continue their growth if a proton-donating reagent is added; each of the resulting dead polymers then contains a C-H end-group. However, if CO<sub>2</sub> is added instead of a proton-donating reagent, an identical polymer is formed which possesses a COOH end-group in place of a C-H end-group. Similarly, the addition of ethylene oxide endows it with a hydroxylic end-group, and still

\* Or, more correctly, when the monomer attains its equilibrium concentration characterizing the monomer-living polymer system (szwarc 1967).

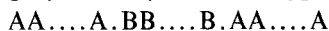
other desired end-groups can be introduced by using other reagents. Hence, a series of polymers may be prepared, identical in every respect except the nature of their end-groups. The electron-transfer initiation (Szwarc 1956) leads to living polymers with both ends growing and thereafter their deliberate termination yields bi-functional polymers such as dicarboxylic acid, diols, etc. Extension of this work led to the preparation of star-shaped polymers—macromolecules with several chains protruding from a common centre. Each of their ends, if so desired, may be again endowed with any required functional end group.

### BLOCK POLYMERS

The capacity of living polymers to start growing again when a suitable monomer is added leads to the synthesis of block polymers—macromolecules in which a long sequence of one monomer, A, is followed by a sequence of another monomer, B, and so on. Thus, a two-block polymer has the structure



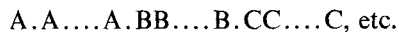
A tri-block polymer may be of the type



or



or



The preparation of block polymers through the living-polymer technique is extremely simple. By 'feeding' a living polymer with monomer A one produces sequences of A's of the desired size and, if necessary, of uniform length. Thereafter, the feeding is continued with another monomer, say B, and a desired sequence of B is added then to the block of A. The only requirement is that a living poly-A is capable of reacting with B. If a living poly-B can initiate the polymerization of A, one can produce a series of polymers of identical size and identical composition, say 50:50 A to B, with any desired distribution of A's and B's along the chain. Hence, instead of random A-B copolymers, organized polymers with a predetermined pattern of monomers may be prepared.

Further simplification of preparation of tri-block polymers, A...A.B...B.A...A, is achieved if a living poly-B with both ends active is used in the synthesis. Feeding such a polymer with monomer A yields at once a tri-block polymer with equally long sequences of A flanking the centre sequence of poly-B.

The behaviour of tri-block polymers, polystyrene-polyisoprene-polystyrene,

or polystyrene-polybutadiene-polystyrene, exemplifies some unusual properties of block polymers. Such polymers prepared in hydrocarbon medium with  $\text{Li}^+$  counter-ions have the properties of vulcanized, i.e. cross-linked, rubber, and the tensile strength of the resulting material at room temperature is appreciable. However, at elevated temperatures the material melts, becomes fluid and can be recast in a new mould; hence, it is referred to as thermoplastic rubber. The high tensile strength is lacking, however, in both a tri-block polybutadiene-polystyrene-polybutadiene and a di-block polystyrene-polybutadiene, even when the composition and molecular weight of these polymers are identical to those of the thermoplastic rubber. We face here a striking manifestation of properties which are determined by the mode of distribution of monomers along the polymer chain.

What endows the thermoplastic rubber with its unusual properties? Its peculiar behaviour is determined by its morphology. As is well known, most of the polymers are incompatible with each other; hence the polystyrene segments of the thermoplastic rubber tend to aggregate, forming domains of polystyrene dispersed in a continuum of the rubbery polybutadiene or polyisoprene. These domains act as giant cross-linking centres when the material is kept below the glass temperature of polystyrene, and thus the properties of vulcanized rubber appear. At higher temperatures the polystyrene domains melt and the cross-linking behaviour is lost. The cross-linking characteristic requires each polybutadiene chain to possess two 'anchors' and therefore the polystyrene-polybutadiene-polystyrene tri-block, and not the polybutadiene-polystyrene-polybutadiene, exhibits the desired properties.

The incompatibility of polymers gives rise to various morphological structures, often referred to as mesomorphic structures, that spontaneously develop in concentrated, gel-like block-polymer solutions. The tendency for blocks of the same kind to become aggregated leads to domains the dimensions of which are determined by the molecular weight of the blocks, as well as by the nature and the amount of the added solvent. The length of the blocks limits the size of the domains. A domain of poly-A cannot be extended further away from its surface than the length of the poly-A block, otherwise a block of poly-B would be pulled into a domain of poly-A. This is precisely what the system tries to avoid.

What are the morphological forms of feasible domains? At least three forms are known: layers containing one kind of blocks alternating with layers built from the others, spheres of blocks A regularly dispersed in a continuum of blocks B, and parallel cylinders of A regularly protruding through the continuum of B. The morphology of the domains is governed by factors such as the nature of the segments and of the solvent, the composition and molecular weight of the block polymers, and the temperature and concentration of the gel.

The parallel layers of poly-A blocks alternating with layers of poly-B blocks seem to represent the simplest morphological structure. Since the layers are of uniform size they act as a diffraction grating, and for a sufficiently high molecular weight, i.e. for sufficiently thick layers, the colourless material acquires an interference colour which may be varied by varying the molecular weight of the

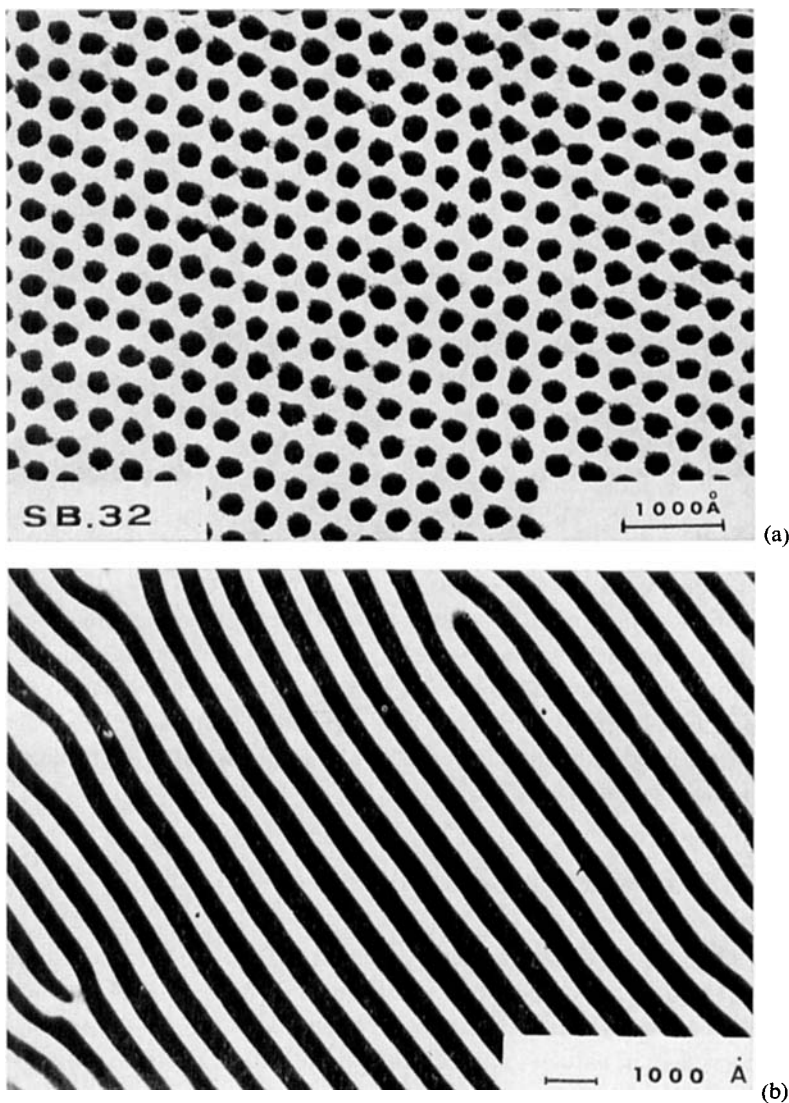


FIG. 1. Electron micrographs of polystyrene-polybutadiene block polymers in rigid medium. Sliced (a) perpendicularly and (b) parallel to the axis of the cylinder. (From Ruckel & Schuerch 1966.)

blocks. When shear takes place the colours change but the material relaxes thereafter and the original colour returns.

Studies of mesomorphic structures of block polymers were initiated by the pioneering investigations of Skoulios *et al.* (1960), Skoulios & Finaz (1961) and Sadron (1963). Di-blocks of polystyrene and polyethylene oxide were the first to be investigated by the low-angle X-ray scatter technique. The gels produced with nitromethane solvent resulted in cylindrical micelles whereas lamellae were observed when butyl phthalate was used as a solvent.

It is also interesting to note that the properties of the materials produced from block polymers after evaporation of the solvent depend on the nature of the solvent. For example, when cast from a solvent good for polystyrene but bad for polybutadiene, a block polymer of polystyrene and polybutadiene yields a film with properties resembling those of polystyrene film. However, a rubbery film is obtained when the material is cast from a solvent good for polybutadiene but poor for polystyrene.

Let us close this section by describing interesting and novel materials prepared by B. Gallot and C. Sadron. Various di-block polymers were prepared by the living-polymer technique and mesomorphic phases were developed using monomers such as styrene or methyl-methacrylate as the solvents. After the mesomorphic phase had been developed the monomeric solvent was polymerized, e.g. photochemically. The gel then becomes converted into a rigid material which may be sliced with a microtome and stained. Two different slices obtained from the same sample are shown in Fig. 1.

Inspection of these figures shows that the block polymers are forming long cylinders of polybutadiene (stained by  $\text{OsO}_4$ ) in a matrix of polystyrene. The slicing is random; however if it happens to be in a plane perpendicular to the cylinder axes a photograph like that shown in Fig. 1a is obtained. On the other hand, if the slicing occurred in a plane parallel to the cylinder axes the resulting photograph looks like Fig. 1b.

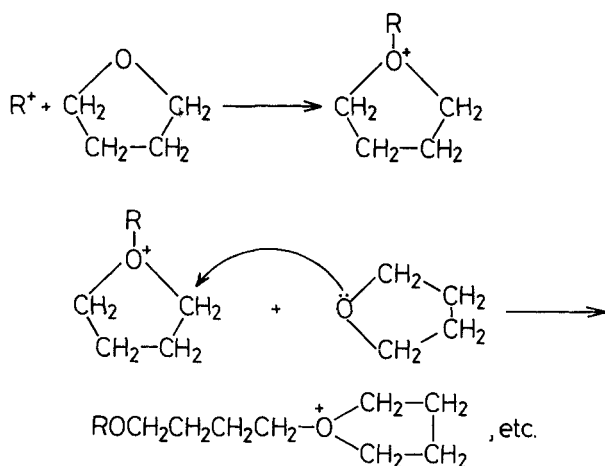
#### SYNTHETIC STEREOSPECIFIC POLYSACCHARIDES

The most challenging field for the application of our knowledge of the mechanisms of stereoregular polymerization and of the nature of reaction intermediates is the synthesis of biopolymers. Of the three major polymer systems found widely in nature—nucleic acids, proteins and polysaccharides—only the polysaccharides require for their synthesis a chain-forming reaction under complete steric control. For this reason alone, their synthesis is perhaps the most difficult.

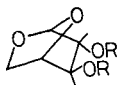
The chain-forming reaction is an acetal synthesis, formally between the hemiacetal function at  $C_{(1)}$  and any one of several different hydroxyl groups on the substituent sugar units. The monomeric sugars are asymmetrical. The hexoses, for example, have four asymmetrical carbon atoms, one asymmetrical hemiacetal function, two possible ring sizes and four sites of linkage between the  $C_{(1)}$  hemiacetal and a hydroxyl group. There are, therefore, 256 stereoregular linear homopolysaccharides theoretically derivable from aldohexoses alone. To control the structure of the polymer produced, the ring size must be fixed and all but one hydroxyl group blocked before the chain-forming reaction is attempted.

By far the best results in terms of high stereospecificity and high molecular weights have been obtained in the cationic-ring-opening polymerization of some anhydro sugar derivatives. To date, three ring types have been investigated, namely 1,2-anhydro, 1,4-anhydro, and 1,6-anhydroglucopyranoses. For a variety of reasons ester groups are not satisfactory blocking groups in these syntheses and ether substituents must be used instead. Since the only known 1,2-anhydro sugar derivatives contain ester functions, their polymerization will not be discussed. The 1,4-anhydro and 1,6-anhydro sugar derivatives polymerize in a manner reminiscent of the cationic polymerization of tetrahydrofuran. However, the asymmetry of these monomers gives more insight into the reaction and it is now apparent that there are well defined although rather narrow conditions within which stereospecific polymerizations can be achieved.

The mechanism usually proposed for tetrahydrofuran polymerization involves trialkyl oxonium ions as intermediates:



This reaction mechanism implies simultaneous bond breaking and bond formation and in the anhydro sugars it should result in stereospecific polymerization. In the 1,4-anhydro sugars two factors interfere. First there is little difference in basicity between the two ring oxygens and little difference in strain between the five- and six-membered rings. As a result, either ring oxygen may be attacked by the propagating ion with the corresponding ring-opening reaction. Therefore both pyranose and furanose rings are incorporated into the chain.



In spite of this complexity both reactions might still proceed stereospecifically. However both  $\alpha$  and  $\beta$  furanosidic structures are in fact found in the polymer and their relative proportions vary with reaction conditions in ways which suggest that the interconversion of a close and a loose ion pair accompanies the polymerization. It is not possible to explain these and other results in terms of a trialkyl oxonium ion intermediate, and one must postulate, therefore, the presence of carbonium ions (see Fig. 2).

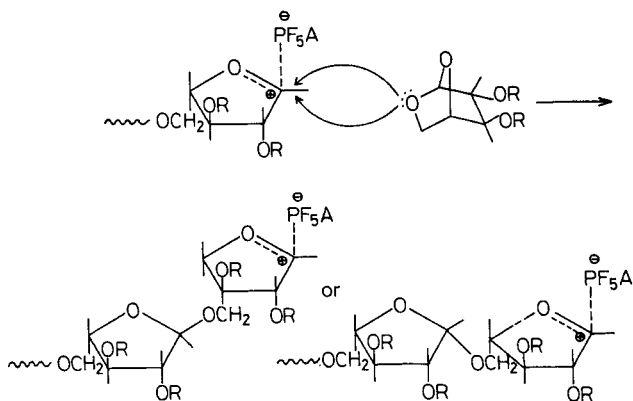


FIG. 2. Propagation by a carbonium ion mechanism.

The 1,6-anhydro sugar derivatives form a fused five- and six-membered ring. The ring-bridging by  $\text{C}_{(6)}$  forces the substituents on  $\text{C}_{(1)}$  and  $\text{C}_{(5)}$  to be in axial conformation. In the anhydro gluco-, manno- and galacto-sugars (all of which have been polymerized), either two or three other alcoholic substituents are also axial. There is therefore substantial driving force from non-bonded interactions leading to the opening of the five-membered ring during polymerization, and the resulting polymers have the pyranose structure.

The stereoregular polymers are those expected from the attack of ring oxygen on  $C_{(1)}$  of the trialkyl oxonium ion which proceeds simultaneously with the bond-breaking process (Fig. 3).

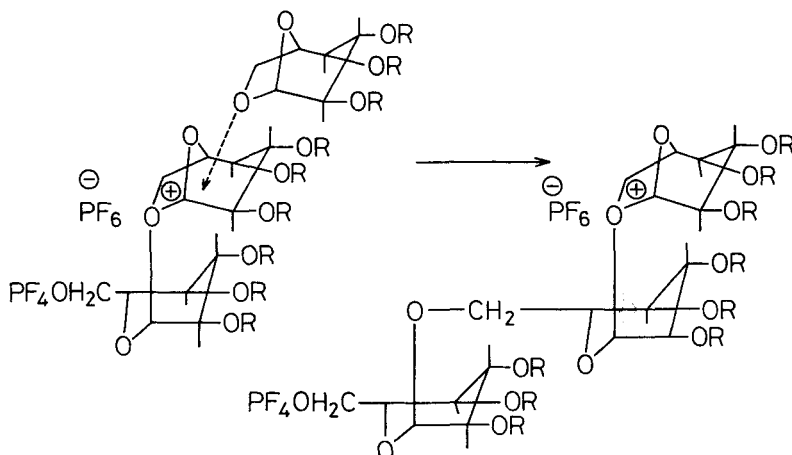


FIG. 3. 1,6-Anhydro ring-opening polymerization; stereospecific activated chain. (From Ruckel & Schuerch 1966.)

Although many of the main features of the polymerizations can be explained in terms of a propagating cation, the counter-ion also plays a role in the process. To date stereoregular polymers have been obtained only with Lewis acids that contain fluorine, i.e. with boron trifluoride, phosphorus pentafluoride and antimony pentafluoride, while antimony pentachloride or tin tetrachloride yield non-stereospecific polysaccharides. The temperature at which the polymerization proceeds is also a function of the counter-ion. Boron trifluoride etherate catalyses stereoregular polymerization at temperatures ranging from  $+25^{\circ}$  to  $-20^{\circ}\text{C}$ ; at lower temperatures the reaction does not occur. Phosphorus pentafluoride initiates stereospecific polymerization at lower temperatures, i.e. from about  $-55^{\circ}$  to  $-78^{\circ}\text{C}$  but above this temperature the reaction is no longer stereoregular. Apparently the stability and reactivity of the propagating ion is a function of the counter-ion.

Most of the work has been carried out with 1,6-anhydro-2,3,4,-tri-O-benzyl- $\beta$ -D-gluco-, -manno-, and galactopyranose. These differ only in their configurations on  $C_{(2)}$  and  $C_{(4)}$  centres. However their rates of polymerization are quite different: manno > gluco >> galacto, and the maximum degrees of polymerization obtained follow the same order: manno > gluco > galacto. The polymers are amorphous because of the bulky aromatic side groups and readily soluble in a variety of solvents. However, the solubilities of the parent polysaccharides,

obtained by debenzoylation with sodium in liquid ammonia, are strikingly different. The glucan polymer is water-soluble with some tendency to crystallize out on standing. The mannan polymer is dispersible and partially soluble in water but requires dimethyl sulphoxide for its complete solution. The galactose polymer is insoluble in essentially all except some complex-forming solvents. This striking difference in solubility results solely from the difference in configuration around a single carbon atom attached to a hydroxyl group and not from a configurational difference at  $C_{(1)}$  which differentiates starch from cellulose. Anhydrodisaccharides can also be polymerized to chains of 14–30 saccharide units, and their copolymerizations proceed normally.

The synthetic linear glucan has a backbone structure identical to that of clinical dextran, which is a slightly branched polysaccharide produced by bacteria and used as a blood volume expander. A similar dextran is implicated in caries formation by oral bacteria. The synthetic glucan has been tested enzymically by two independent laboratories and found to be 100% stereoregular and about 98%  $\alpha$ 1,6. Similar results have been obtained on the mannan. Failure to precipitate conconavalin A confirmed the linearity of both these synthetic polysaccharides. The mannan and galactan are unknown in nature except as fragments of more complex polysaccharides from yeast and fungi. A related  $\alpha$ 1,6-linked mannan with  $\alpha$ 1,2-linked mannose side chains is formed by a dermatophyte and causes allergic reactions. Similar allergenic effects are observed with the synthetic mannan and this unequivocally demonstrates that these reactions are induced by carbohydrate and not by protein or nucleic acid impurities. A similar mannan has also been reported to be active against viral infections by activating interferon production and to have caused regression of sarcoma 180 in experimental animals.

It is therefore obvious that these polysaccharides serve a useful function as model substances in immunological, serological, enzymic and pharmaceutical investigations. They also permit the systematic investigation of structure-property relationships.

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## Discussion

*Katchalski*: Why don't the various types of polymerization that you described, such as ionic polymerization and the coordination type, appear in biological systems? Is it because these types cannot occur in water?

*Szwarc*: That is not the only reason. Some of these polymerizations may proceed in aqueous solution. However, most of the monomers I discussed never appear in biological systems. For example, no isoprene is present in a rubber tree and biosynthesis of rubber proceeds through different intermediates than in its laboratory or industrial synthesis.

*Katchalski*: You indicated that some of your polymerization techniques lead to products possessing sharp molecular weight distributions of the Poisson type, yet the biologist is familiar with a sharper type of distribution where every molecule is of equal weight. Of course there are biopolymers such as polysaccharides which display polydispersity, but in most cases all the molecules of the biopolymer, such as those of a given protein or nucleic acid, are of the same size. Formation of molecules of the same size and structure is determined *in vivo* by a template mechanism. Can you suggest any other mechanisms for obtaining molecules of identical size?

*Szwarc*: The template mechanism usually operates whenever polymers of the type A-B-C-D- are produced, i.e. when there is a non-uniform pattern in the resulting polymer. The pattern as well as the size have to be determined by a template and hence the resulting polymer has a unique size. The biological way of achieving such a template polymerization is well known and it is unnecessary for me to elaborate this topic.

A synthetic template polymerization has been attempted in Germany (see

Kämmerer & Ozaki 1966). The idea was to produce a polyol, esterify it with acrylic acid and polymerize the vinyl monomer attached to such a 'template'.

*Changeux*: Could you make polymers of the compounds referred to as photochromes whose properties change when exposed to light?

*Szwarc*: Yes. Prins (1971), for example, prepared gels which contain chromophoric groups, which upon irradiation isomerize and then soak up more water and therefore swell. In the dark the original isomer is re-formed and the gels let the water out. Smets (1968) has synthesized polymers with stilbene units in the chain. On irradiation the *trans* form isomerizes to the *cis*, and this substantially modifies the conformation of the polymer and therefore its structure and properties. Again, there is a relaxation which takes place in the dark.

*Luzzati*: The structural work on these systems originated from the study of lipids. The analogy is obvious. Lipid molecules consist of hydrocarbon chains and polar groups: the two moieties have different solubilities. This led us to try block copolymers in the presence of solvent, with the hope that the two regions would segregate according to their affinity with the solvent. Indeed several structures were obtained, which we had found previously in lipid-water systems.

The problem of monodispersity is quite interesting. In the field of proteins and nucleic acids we are now accustomed to dealing with monodisperse compounds, with a specific and unique chemical structure. But it is not always like that in biology: membranes are an interesting example where the lipids are highly polydisperse. In spite, or perhaps because, of that polydispersity, and a high disorder in the short-range conformation, lipids can display a large number of highly ordered phases.

*Engel*: Is the equilibrium in living polymers always on the side of the polymer, Dr Szwarc? Is there a difference in equilibrium constant between the binding of A to B and the binding of equal segments? That is, do you have to consider a redistribution of A and B in your polymers?

*Szwarc*: I presume that you are enquiring about the equilibrium between a living polymer and its monomer. A living polymer containing  $n$  units may add its monomer and yield an  $n+1$ -mer. According to the principle of microscopic reversibility the  $n+1$ -mer may degrade and yield an  $n$ -mer and monomer. Therefore, by virtue of the fact that the polymers are living, in my meaning of the term, they degrade as well as grow and therefore the conversion of monomer into polymer never goes to completion. Thus, the system attains a state of equilibrium at a concentration of monomer at which the rate of propagation is exactly balanced by the rate of depropagation. Living polymer is then in equilibrium with its monomer. One should ask, what is this equilibrium monomer concentration? That depends on the nature of the monomer, on the