Hans Bisswanger

Enzyme Kinetics

Principles and Methods

Second, Revised and Updated Edition

WILEY-VCH Verlag GmbH & Co. KGaA

Hans Bisswanger
Enzyme Kinetics

Related Titles

Bisswanger, H.

Practical Enzymology

2004
ISBN: 978-3-527-30444-8

Aehle, W. (Ed.)

Enzymes in Industry
Production and Applications

2007
ISBN: 978-3-527-31689-2

Reymond, J.-L. (Ed.)

Enzyme Assays
High-throughput Screening, Genetic Selection and Fingerprinting

2006
ISBN: 978-3-527-31095-1

Breslow, R. (Ed.)

Artificial Enzymes

2005
ISBN: 978-3-527-31165-1

Buchholz, K., Kasche, V., Bornscheuer, U.T.

Biocatalysts and Enzyme Technology

2005
ISBN: 978-3-527-30497-4

Hans Bisswanger

Enzyme Kinetics

Principles and Methods

Second, Revised and Updated Edition

WILEY-VCH Verlag GmbH & Co. KGaA

The Author

Prof. Dr. Hans Bisswanger
Interfakultäres Institut für Biochemie
Hoppe-Seyler-Str. 4
72076 Tübingen
Germany

1st edition translated by Leonie Bubenheim.

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Bibliographic information published by the Deutsche Nationalbibliothek
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet at http://dnb.d-nb.de.

Composition K+V Fotosatz GmbH, Beerfelden
Printing Betz-Druck GmbH, Darmstadt
Bookbinding Litges & Dopf GmbH, Heppenheim

Printed in the Federal Republic of Germany
Printed on acid-free paper

ISBN: 978-3-527-31957-2

For Anna and Michael

Preface to the Second English Edition

Die Zeit, innerhalb welcher eine bestimmte Menge Substrat verändert wird, also das Maß der Reaktionsbeschleunigung durch den Katalysator, hängt in erster Linie von seiner Menge ab. In sehr vielen Fällen ist sie sogar direkt proportional der wirksamen Menge des Fermentes, in anderen Fällen bestehen kompliziertere Beziehungen, die man in den sogenannten „Fermentgesetzen" hat ausdrücken wollen, die aber zum großen Teil sehr mangelhaft fundiert sind.

The time needed for a distinct amount of substrate to be changed, hence the degree of acceleration of the reaction by the catalyst, depends primarily on its amount. In a great number of cases it is even directly proportional to the efficient amount of the ferment. In other cases more complicated relationships exist. It was attempted to formulate these in the so-called "ferment laws". However, to a large extent, they are very insufficiently substantiated.

Carl Oppenheimer (1919) *Biochemie*
Georg-Thieme-Verlag Leipzig

Upon preparing a new edition of a book inevitably the question arises: what should be changed? A textbook is bound to present the fundamental topics, which cannot easily be modified without disturbing the whole concept and, since enzyme kinetics is not a very expanding area of biochemistry, most topics can be regarded as fundamental. One challenging area not satisfactorily covered by classical enzyme kinetics concerns enzymes located at boundary layers, especially at membranes. Actually, about half of the enzymes in a cell are in more or less intense contact with membranes. Such enzymes and their reactions are different to reactions in aqueous solutions and cannot be treated in the same way because of profound differences in the immediate environment, the access of substrates and the release of products. The situation is complicated by the fact that there exists no general mode of membrane association of enzymes. While various enzymes are completely integrated into the membrane, others are more or less loosely associated. Further, it must be considered that (non-enzymatic) transport processes through membranes also exhibit similarities with enzyme reactions, translocation from one site to another in the membrane can be compared with the conversion of substrate to product. This opens a broad field for the extension of the rules of enzyme kinetics. However, since such sys-

tems cannot satisfactorily be described by one or a few general rules, special mechanisms have to be developed for each individual system; the discussion of this topic is restricted to prominent systems, immobilized enzymes, membrane integrated enzymes and transport systems, and enzymes on boundary layers between the aqueous solution of the cytosol and the membrane. A striking conformity of all these systems is the broad applicability of the basic equation of enzyme kinetics, the Michaelis-Menten law which, as a rule, can be used in a first approach, while the special features of the respective system may necessitate distinct modifications. The concept of allosteric enzymes is extended to membrane systems. Nevertheless, only suggestions can be given, while for distinct solutions for a certain system the special literature must be consulted.

In a separate section a comparison is drawn between enzyme kinetics and pharmacokinetics, two closely related areas, both depending on enzyme reactions and thus sharing various similarities. However, there are also essential differences, enzyme kinetics treating defined enzyme reactions, while pharmacokinetics deals with more complex processes observing the fate of a drug during its journey through the organism. The different terminology employed in these fields is compared to facilitate mutual understanding.

The text was generally revised especially in order to simplify the understanding of the theoretical, often dry, matter. Sections not directly required for the continuous treatise are set in separate boxes.

I thank Mr. Zhougang Yang for updating and designing many of the figures.

Tübingen, January 2008 *Hans Bisswanger*

Preface to the First English Edition

The time about three decades ago may be regarded as the *Golden Age* of enzyme kinetics. Then it became obvious that many biological processes can be forced into terrifying formulas with which experts intimidate their colleagues from other fields. The subject has been treated in several competent textbooks, all published in the English language.

For students with English not being their mother tongue this did not provide a simple language problem, but rather confronted them simultaneously with a difficult matter *and* a foreign language. So the original intention to write a textbook in German was to minimise the fear of the difficult matter. Very difficult derivations were renounced realising the fact that most biochemists will never need or keep in mind every specialised formula. They rather require fundamentals and an understanding of the relationships between theoretical treatments and biological processes explained by such derivations as well as the knowledge which practical approaches are most suited to examine theoretical predictions. Therefore, the book is subdivided into three parts, only the central chapter dealing with classical enzyme kinetics. This is preceded by an introduction into the theory of binding equilibria and followed by a chapter about methods for both binding studies and enzyme kinetics including fast reactions.

Since the German edition is well introduced and the concept broadly accepted, the publication of an English edition appeared justified. This is supported by the fact that new editions in enzyme kinetics are rare, although a thorough understanding of the field as an essential branch of biochemistry is indispensable. The original principle of the former editions to present only fundamentals for a general understanding cannot consequently be maintained, as a specialist book on the subject must exceed the level of general textbooks and should assist the interested reader with comprehensive information to solve kinetic problems. Nevertheless, the main emphasis still is to mediate the understanding of the subject. The text is not limited to the derivation and presentation of formula, but much room is given for explanations of the treatments, their significance, applications, limits, and pitfalls. Special details and derivations turn to experts and may be skipped by students and generally interested readers.

The present English edition is a translation of the Third German edition including revisions to eliminate mistakes.

I would like to acknowledge many valuable suggestions especially from students from my enzyme kinetics courses as well as the support from WILEY-VCH, especially from Mrs. Karin Dembowsky. Her encouraging optimism was a permanent stimulus for this edition.

Tübingen, January 2002 Hans Bisswanger

Contents

Symbols and Abbreviations

Units indicated in brackets.
Special, rarely used abbreviations are defined in the text.

A, B, C	ligands, substrates
[A], [B], [C]	concentration terms for ligands/substrates (similarly for enzymes, products, inhibitors etc.)
A	absorption measure
AUC	area under the curve
B	absolute bioavailability
c	concentration
CL	clearance
D	diffusion coefficient
e	Euler number (e=2.71828)
E	enzyme, macromolecule
E_a	activation energy
EC_{50}	effective concentration
F	relative intensity of fluorescence
FRET	fluorescence resonance energy transfer
ΔG°	standard Gibbs energy
G	electric conductance (S)
ΔH°	standard reaction enthalpy
h	Planck constant (6.626×10^{-34} Js)
h_s	transport coefficient of substrate
I	inhibitor
I	light intensity
IC_{50}	inhibitory concentration
J	flux (e.g. of a ligand from one compartment to another)
IU	enzyme unit (international unit, μmol min^{-1}, 1 IU = 16.67 nkat, 1 nkat = 0.06 IU)
K	microscopic equilibrium constant
K'	macroscopic equilibrium constant
K_a	association constant
K_{app}	apparent equilibrium constant
K_d	dissociation constant
K_g	equilibrium constant of a reaction
K_i	inhibition constant
K_{ic}	competitive inhibition constant
K_{iu}	uncompetitive inhibition constant
K_m	Michaelis constant
K_{mA}	Michaelis constant for substrate A
$k_{1,2,3\ldots}$	rate constant in forward direction
$k_{-1,-2,-3\ldots}$	rate constant in reverse direction
k_{cat}	catalytic constant
k_B	Boltzmann constant (k_B=R/N=1.3810^{-23} JK^{-1})
kat	Katal, enzyme unit according to the SI system (mol s^{-1})
M	amount of the drug applied
M_r	relative molecular mass (dimensionless)
m	number of binding classes per macromolecule
n	number of identical binding sites per macromolecule
n_h	Hill coefficient
N_A	Avogadro constant (6.022×10^{23} mol^{-1})
Or	ordinate intercept
P, Q, R	products

P	polarization
R	gas constant (8.314 J K^{-1} mol^{-1})
r	fraction of ligands bound per macromolecule
ΔS	standard reaction entropy
Sl	slope
T	absolute temperature (K)
t	time (s)
U	voltage (V)
v	reaction velocity
v_0	initial velocity for $t=0$
V	maximal velocity for substrate concentrations $\rightarrow \infty$
V_d	distribution volume
$\bar{Y}$	fraction of ligands bound per binding site
a	normalized ligand concentration $[A]/K_d$
ε	molar absorption coefficient
Φ	optical rotation
Φ_F	quantum yield
Φ_s	substrate resp. Thiele module
η	viscosity
η_e	efficiency factor
η_{e1}	efficiency factor for first order reactions
λ	wavelength (nm)
Θ	ellipticity
ρ	density (kg m^{-3})
τ	relaxation time

Introduction and Definitions

In the simplest form an enzyme reaction can be formulated:

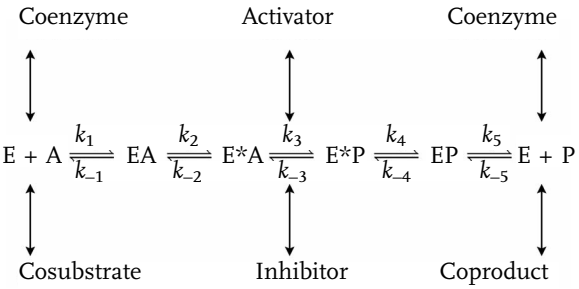
$$E + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA \xrightarrow{k_2} E + P \,.$$

A substrate A reacts with an enzyme E to form an enzyme–substrate complex EA, which is converted to product in an irreversible step, releasing the free enzyme, which enters into a new reaction cycle. The single reaction steps are designated by rate constants k, marked with positive figures in the forward direction (k_1, k_2, $k_{3\ldots}$) and with negative figures in the backward direction (k_{-1}, k_{-2}, $k_{-3}\ldots$). A more detailed examination of even such an apparently simple reaction requires the consideration of more steps:

$$E + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E^*A \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E^*P \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} EP \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} E + P \,.$$

After initial binding of the substrate to the enzyme a loose association complex EA is formed in a rapid equilibrium. In the next step the bound substrate is adapted to a strong transition state by interaction with the residues of the catalytic center E*A and is prepared for conversion to the product E*P. Before the formation of free product P and enzyme an association complex EP is formed. The whole reaction sequence appears quite symmetrical and can be run through from both the forward and backward direction (P acting as substrate and A as product). Now five equilibria are considered and, for a complete description of the same reaction, ten rate constants must be known, instead of only the three assumed in the first scheme.

Actually the situation is even more complicated as, for most enzyme reactions, more components must be considered, such as two or more substrates (A, B, C…) and products (P, Q, R…), cofactors, inhibitors and activators (the latter two are also called *effectors*):



It is obvious that such complex mechanisms cannot be explored by only one or even a few experiments. Sophisticated theoretical treatments and extensive methodical approaches are required to unravel these mechanisms. To simplify the scheme, the various steps can be assigned to two different types, equilibrium and kinetic steps, and steps of each particular type can be treated similarly. Binding studies for the different ligands must be undertaken to investigate individual equilibria and in combination with kinetic studies and studies of conformational changes the central steps can be elucidated. Considering all the information the complete mechanism can be derived from the detailed results.

The investigation of binding equilibria requires the strict exclusion of any kinetic processes and is, in principle, different to kinetic studies, but it is an indispensable contribution to the understanding of enzyme mechanisms. Consequently, before treating enzyme kinetics in detail, equilibria will be discussed. Because of the strict absence of kinetic processes, this treatment is not restricted only to enzymes, but is applicable to any specific binding process and, since binding is understood usually to be the specific interaction of a low-molecular weight compound with a larger target, like a protein, receptor, DNA, etc., in this chapter the term *macromolecule* will be used synonymously with the term enzyme (both designated with E), while the following chapters on enzyme kinetics will be confined to enzymes. The low-molecular weight binding compound, which may be a substrate, an inhibitor, activator, hormone or any other specific binding metabolite, is called a *ligand*. It is assumed that there exists a distinct region for the ligand on the macromolecule, a specific *binding site*, in contrast to unspecific binding, due for example to ionic or hydrophobic interactions. The various mechanisms for equilibria between different ligands and macromolecules are gathered together under the term *multiple equilibria*.

The principal differences between equilibrium and kinetic investigations are summarized in Table 1. Equilibria are obviously time-independent and, therefore, the determination methods do not depend on time, measurements can be performed for long periods, while kinetic measurements are confined to the limited time when the reaction proceeds. In reality, however, it must be considered that biological substances, like enzymes, are not very stable, especially under experimental conditions and, therefore, equilibrium measurements should also be performed within a short time. A severe disadvantage of equilibrium measurements is that reversible binding causes no real change in the features

Table 1 Differences between equilibrium and kinetic studies.

Procedure	Equilibrium studies	Enzyme kinetic studies
Time dependence	Time independent rapid equilibrium	Time dependent directional progression
Constants	Thermodynamic constants: dissociation (association) constants	Kinetic constants: Michaelis constant, maximum velocity
Detection principal	Free and bound components chemically identical	Substrate and product chemically different
Detection sensitivity	Dependent on macromolecule concentration	Dependent on product formation
Macromolecule/enzyme amounts required	Macromolecule and ligand in comparable amounts $[E] \sim [A]$	Catalytic enzyme amounts $[E] \ll [A]$
Purity requirements	Macromolecule present in high purity	High enzyme activity, no requirement for high purity if no disturbing influences

of the components, while enzyme reactions proceed with chemical conversion of substrate to product, which can be used as a detection signal. Therefore, there is no need for high amounts of enzymes in enzyme kinetics, while with binding measurements the amount of bound ligand is directly proportional to the macromolecule concentration, which must be considerably high to enable accurate detection. The requirements for purity of the enzyme or the macromolecule are also different. For binding measurements high purity is usually needed, because the molar concentration must be known, while for enzyme kinetic determinations only disturbing influences, like side reactions, must be eliminated and inert contaminations are mostly unobjectionable. Finally, there are also major differences in the constants. From equilibrium treatments thermodynamic constants, like association or dissociation constants, are derived, while kinetic studies yield the more complex kinetic constants. On the other hand there are also similarities. Both types of constant are composed of rate constants, which are valid for both approaches, for example, inhibition constants, although determined kinetically, are really dissociation constants. Generally it can be stated that equilibrium studies are easier to treat theoretically but the experimental procedures are more difficult, while enzyme kinetics has a more complex theory but is easier to handle experimentally and, therefore, is more frequently applied.

Another main area is the treatment of fast reactions. This field can also be differentiated into kinetic methods which directly observe fast reactions, i.e. the continuous and stopped flow methods, while other techniques, like relaxation methods, deal with equilibria (although the deviation from equilibrium is a kinetic process). These techniques allow one to analyse complex mechanisms from a particular viewpoint and, besides the fact that fast processes become accessible, distinct rate constants can also be determined, rendering this approach

as a valuable completion of equilibrium and kinetic studies with conventional methods.

Although different areas are treated in this book a uniform nomenclature is used throughout. For example, equilibrium treatments are connected to thermodynamics and deal mostly with association constants, while enzyme kinetics involve dissociation constants (the Michaelis constant is related to a dissociation constant), both types of constants describe principally the same equilibrium, only in a reversed sense. Since the main emphasis of this book is on enzyme kinetics, dissociation constants are used throughout. The terms A, B, C… will be used for any ligand, including substrates, binding specifically to a macromolecule or an enzyme. Only if it is necessary to discriminate between distinct types of ligands will different terms be used, e.g. I for the inhibitors, P, Q, R… for products. As far as possible the NC-IUB recommendations (Nomenclature Committee of the International Union of Biochemistry, 1982) and the IUPAC rules (International Union of Pure and Applied Chemistry, 1981) are regarded. Concentrations are indicated by square brackets ([A], etc.). The following reference list comprises standard textbooks relevant to the various fields treated in this book but is not a complete list of all such books.

References

Bisswanger, H. (2004) *Practical Enzymology*, Wiley-VCH, Weinheim.

Cornish-Bowden, A. (1976) *Principles of Enzyme Kinetics*, Butterworth, London, Boston.

Cornish-Bowden, A. (2004) *Fundamentals of Enzyme Kinetics*, 3rd edn, Portland Press Ltd. London.

Cornish-Bowden, A., Wharton, C.W. (1988) *Enzyme Kinetics*, IRL Press, Oxford.

Dixon, M., Webb, E.C. (1979) *Enzymes*, Academic Press, New York.

Edsall, J.T., Gutfreund, H. (1983) *Biothermodynamics*, J. Wiley & Sons, New York.

Eisenthal, R., Danson, J.M. (1992) *Enzyme Assays. A Practical Approach*, IRL Press, Oxford.

Engel, P.C. (1996) *Enzymology Labfax*, Academic Press, New York.

Fersht, A. (1977) *Enzyme Structure and Mechanism*, W.H. Freeman & Co., San Francisco.

Fromm, H.J. (1975) *Initial Rate Kinetics*, Springer, Berlin.

Johnson, K.A. (2003) *Kinetic Analysis of Macromolecules: A Practical Approach*, Oxford University Press, Oxford.

Klotz, I.M. (1986) *Introduction to Biomolecular Energetics Including Ligand–Receptor Interactions*, Academic Press, Orlando.

Kuby, S.A. (1991) *Enzyme Catalysis, Kinetics and Substrate Binding*, CRC Press, Boca Raton.

Laidler, K.J., Bunting, P.S. (1973) *The Chemical Kinetics of Enzyme Action*, 2nd edn, Clarendon Press, Oxford.

Leskovac, V. (2003) *Comprehensive Enzyme Kinetics*, Kluwer Academic, Dordrecht.

Marangoni, A.G. (2003) *Enzyme Kinetics. A Modern Approach*, Wiley-Interscience, Hoboken, New Jersey.

Page, M. (Ed.) (1984) *The Chemistry of Enzyme Action. New Comprehensive Biochemistry*, Vol. 6. Elsevier, Amsterdam.

Price, N.C., Stevens, L. (1989) *Fundamentals of Enzymology*, Oxford University Press, Oxford.

Purich, D. L. (Ed.) (1982) *Enzyme Kinetics and Mechanism, Methods in Enzymology,* Vol. 87, Academic Press, New York.
Purich, D. L. (1996) *Contemporary Enzyme Kinetics and Mechanism,* Academic Press, New York.
Purich, D. L. (1999) *Handbook of Biochemical Kinetics,* Academic Press, New York.
Roberts, D. V. (1977) *Enzyme Kinetics,* Cambridge University Press, Cambridge.
Segel, I. H. (1975) *Enzyme Kinetics,* J. Wiley & Sons, New York.
Taylor, K. B. (2002) *Enzyme Kinetics and Mechanisms,* Kluwer Academic Publishers, Dordrecht, NL, Boston, London.

Nomenclature rules

International Union of Pure and Applied Chemistry (1981) *Symbolism and terminology in chemical kinetics, Pure Appl. Chem. 53,* 753–771.
Nomenclature Committee of the International Union of Biochemistry (1982) *Symbolism and terminology in enzyme kinetics, Eur. J. Biochem. 128,* 281–291.

1
Multiple Equilibria

Chemical reactions are initiated by accidental collision of molecules, which have the potential (e.g. sufficient energy) to react with one another to be converted into products:

$$A + B \longrightarrow P + Q$$

In living matter it cannot be left to chance whether a reaction happens or not. At exactly the time required the respective compounds must be selected and converted to products with high precision, while at unfavorable times spontaneous reactions must be prevented. An important prerequisite for this selectivity of reactions is the highly specific recognition of the required compound. Therefore, any physiological reaction occurring in the organism is preceded by a specific recognition or binding step between the respective molecule and a distinct *receptor*. The exploration of binding processes is important for understanding biological processes. The receptors can be enzymes, but also non-enzymatic proteins like membrane transport systems, receptors for hormones or neurotransmitters, and nucleic acids. Generally, receptors are macromolecular in nature and thus considerably larger than the efficacious molecules, the *ligands*. For the binding process, however, they must be treated as equivalent partners (unlike for enzyme kinetics, where the enzyme as catalyst does not take part in the reaction).

As a precondition for binding studies specific binding must be established and unspecific association excluded. There exist many reasons for unspecific binding, like hydrophobic or electrostatic interactions (charged ligands can act as counterions for the surplus charges of proteins). A rough indicator for specific binding is the magnitude of the dissociation constant, which is mostly below 10^{-3} M (although there are exceptions, like the binding of H_2O_2 to catalase or glucose to glucose isomerase). Specific binding is characterized by a defined number of binding sites n, which is in stoichiometric relationship to the macromolecule. In contrast, unspecific binding has no defined number of binding sites, and thus the binding process is not saturable. Furthermore, the ligand can be replaced by structural analogs, while different or distantly related compounds are not accepted.

In the following the processes leading to a specific interaction between a ligand and a macromolecule will be described, i.e. how the ligand finds its bind-

ing site and which factors determine the affinity. The essential mechanisms of interaction between ligand and macromolecule are then presented.

1.1 Diffusion

A prerequisite for any reaction of a ligand with a macromolecule is the fact that the partners must find one another. In a free space a particle moves in a straight direction with a kinetic energy of $k_BT/2$, T being the absolute temperature and k_B the Boltzmann constant. According to Einstein's relationship a particle with mass m, moving in a distinct direction with velocity v possesses kinetic energy $mv^2/2$. Combining both relationships Eq. (1.1) follows:

$$v^2 = k_B T/m \; . \tag{1.1}$$

Accordingly, a macromolecule like the lactate dehydrogenase ($M_r = 140\,000$) would move at a rate of 4 m s^{-1}, its substrate lactic acid ($M_r = 90.1$) at 170 m s^{-1}, and a water molecule ($M_r = 18$) at 370 m s^{-1}. Enzyme and substrate will fly past one another like rifle bullets. In the dense fluid of the cell, however, the moving particles are permanently hampered and deflected from linear movement by countless obstacles: water molecules, ions, metabolites, macromolecules and membranes and, actually, the molecule moves more like a staggering drunkard than in a straight progression. However, this tumbling increases the collision frequency and the probability of distinct molecules meeting one another.

The distance x covered by a molecule in solution within time t in one direction depends on the diffusion coefficient D according to the equation:

$$x^2 = 2Dt \; . \tag{1.2}$$

The diffusion coefficient is itself a function of the concentration of the diffusing compound, in dilute solutions it can be regarded as constant. It depends on the particle size, the consistency of the fluid and the temperature. For small molecules in water the coefficient is $D = 10^{-5}$ cm^2 s^{-1}. A cell with the length 1 μm will be passed within 0.5 ms, 1 mm within 500 s, thus, for a thousandfold distance a millionfold time is required. This demonstrates that there exists no 'diffusion velocity', the movement of the molecules is not proportional to time, but to its square root. A diffusing molecule does not remember its previous position, it does not strive systematically for new spaces but searches new regions randomly in undirected movement. As an example, a 10 cm high saccharose gradient, used in ultracentrifugation for separation and molecular mass determination of macromolecules, has a life-span of about four months, taking $D = 5 \times 10^{-6}$ cm^2 s^{-1} for saccharose. The tendency of the gradient to equalize its concentration is considerably low.

Equation (1.2) describes the one-dimensional diffusion of a molecule. For diffusion in a three-dimensional space over a distance r the diffusion into the three space directions x, y and z is assumed to be independent of each other:

$$r^2 = x^2 + y^2 + z^2 = 6Dt\,. \tag{1.3}$$

Mere meeting of ligand and macromolecule is not sufficient to accomplish specific binding, rather the ligand must locate the binding site on the macromolecule. This is realized by translocation of the ligand volume $4\pi R^3/3$ by the relevant distance of its own radius R. After a time t_x the molecule has searched (according to Eq. (1.3) for $r = R$) a volume of:

$$\frac{6Dt_x}{R^2} \cdot \frac{4\pi R^3}{3} = 8\pi DRt_x\,. \tag{1.4}$$

The volume searched per time unit is $8\pi DR$, the probability of collision for a certain particle in solution is proportional to the diffusion coefficient and the particle radius.

At the start of a reaction $A + B \rightarrow P$ both participants are equally distributed in solution. Within a short time, molecules of one type, e.g. B, become depleted in the vicinity of the molecule of the other type (A) not yet converted, so that a concentration gradient will be formed. Consequently, a net flow Φ of B-molecules occurs in the direction of the A-molecules located at a distance r,

$$\Phi = \frac{dn}{dt} = DF\frac{dc}{dr}\,, \tag{1.5}$$

n is the net surplus of molecules passing through an area F within time t, c is the concentration of B-molecules located at a distance r from the A-molecules. This relationship in its general form is known as *Fick's First Law of Diffusion*. In our example of a reaction of two reactants, F has the dimension of a spherical surface with the radius r. Eq. (1.5) then changes into:

$$\left(\frac{dc}{dr}\right)_r = \frac{\Phi}{4\pi r^2 D'} \tag{1.6}$$

D' is the diffusion coefficient for the relative diffusion of the reactive molecules. Integration of Eq. (1.6) yields:

$$c_r = c_\infty - \frac{\Phi}{4\pi r D'} \tag{1.7}$$

where c_r is the concentration of B-molecules at the distance r and c_∞ the concentration at infinite distance from the A-molecules. The last corresponds approximately to the average concentration of B-molecules. The net flow Φ is proportional to the reaction rate and that is again proportional to the average concentration c of those B-molecules just in collision with the A-molecules, r_{A+B} being the sum of the radii of an A- and a B-molecule:

$$\Phi = kc_{r_{A+B}}\,. \tag{1.8}$$

where k is the rate constant of the reaction in the steady-state, where c_r becomes equal to $c_{r_{A+B}}$ and r equal to r_{A+B}. Inserted into Eq. (1.7), this becomes:

$$c_{r_{A+B}} = \frac{c_\infty}{1 + \dfrac{k}{4\pi r_{A+B} D'}} \,. \tag{1.9}$$

The net flow under steady-state conditions is:

$$\Phi = k_a c_\infty \tag{1.10}$$

where k_a is the relevant association rate constant. Equations (1.8)–(1.10) may thus be rewritten:

$$\frac{1}{k_a} = \frac{1}{4\pi r_{A+B} D'} + \frac{1}{k} \,. \tag{1.11}$$

This relation becomes linear in a graph plotting $1/k_a$ against the viscosity η of the solution as, according to the *Einstein-Sutherland Equation,* the diffusion coefficient at infinite dilution D_0 is inversely proportional to the friction coefficient f and that again is directly proportional to the viscosity η:

$$D_0 = \frac{k_B T}{f} = \frac{k_B T}{6\pi\eta r} \,. \tag{1.12}$$

$1/k$ is the ordinate intercept. In the case of $k \gg 4\pi r_{A+B} D'$ the intercept is placed near the coordinate base, it becomes:

$$k_a = 4\pi r_{A+B} D' \,. \tag{1.13}$$

This borderline relationship is known as the *Smoluchowski limit* for translating diffusion, the reaction is *diffusion-controlled.* In contrast to this, in *reaction-controlled* reactions the step following diffusion, i.e. the substrate turnover, determines the rate. A depletion zone emerges around the enzyme molecule, as substrate molecules are not replaced fast enough. A *diffusion-limited dissociation* occurs, if the dissociation of the product limits the reaction. Viewing two equally reactive spheres with radii r_A and r_B and diffusion coefficients D_A and D_B, we obtain for Eq. (1.13):

$$k_a = 4\pi r_{A+B} D' = 4\pi (r_A + r_B)(D_A + D_B) \,. \tag{1.14}$$

By inserting Eq. (1.12) and with the approximation $r_A = r_B$ and with $D_0 = D_A = D_B$ we obtain:

$$k_a = \frac{8 k_B T}{3\eta} \,. \tag{1.15}$$