A BRIEF HISTORY OF ENZYME KINETICS

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Unlike a well-known scientific book with a title that begins with the same three words, the present article really is brief and really is a history — it actually gives a few dates. In the space available I can do no more than give a broad outline of the development of enzyme kinetics. To give a more detailed account a good deal of mathematics would be necessary. This article will include no mathematical equations, and only a few simple chemical ones.

Every science has two distinct but closely-related aspects: structure, and speed. Fermentation, with which Buchner's name is firmly associated and which has inspired the present volume, provides a simple example. To understand fermentation properly we need to have knowledge of the structures of the chemical substances involved, and of the speeds with which they undergo chemical reaction. Fermentation is an example of a vast group of chemical reactions that are catalysed by enzymes, all of which are protein molecules. The word *catalyst* was coined in 1836 by the great Swedish chemist Jöns Jakob Berzelius, who collected a number of examples of catalysis, though, as noted in the previous chapter of this book (pp. 117–120), he did not discover the concept of catalysis. Almost all of the chemical processes that occur in biological systems would occur extremely slowly if suitable enzymes were not present to speed them up; life as we know it would be impossible.

The matter of the speed with which processes occur is of paramount importance as far as living systems are concerned — indeed as far as the continued existence of the universe is concerned. The branch of chemistry which deals with the rates of chemical processes is known as chemical kinetics, or as chemical dynamics. The branch that deals with the question of whether processes can occur at all is known as chemical thermodynamics. The laws of thermo-

dynamics allow a vast number of chemical reactions to occur, but if there were no restrictions on them the universe as we now know it would exist for no more than a fraction of a second. The restrictions that exist take the form of energy barriers, which chemists call activation energies. If a chemical reaction had zero activation energy, every time two reactant molecules came together there would be instant reaction. If this were true for every possible chemical reaction, there would at once be chaos. Suppose that the world were as it now is, and the barriers were all removed. Every tree would at once react with the oxygen of the atmosphere, and the same would happen to our bodies. In other words, the universe as we know it is as much controlled by the laws of chemical dynamics as by the laws of chemical thermo-dynamics.

Chemists have been making a serious study of the rates of reactions, and the factors that control them, since the middle of the 19th century. Often they found that the rates were proportional to the concentrations of the substances that were reacting together. In 1892, however, the British chemist Adrian John Brown, found in a study of the rate of fermentation of sucrose in the presence of yeast that the rate seemed to be independent of the amount of sucrose present (Brown, 1892). Later he suggested that this result could be explained if the invertase molecules present in the yeast formed an addition complex with the sucrose (Brown, 1902).

This was the first time that the existence of an enzyme–substrate complex was deduced from the kinetics of an enzyme reaction. It was not the first time the idea had been suggested. Brown himself mentioned in his 1902 paper that the distinguished French chemist Charles Adolph Wurtz (1880) had found that papain appears to form an insoluble compound with fibrin prior to hydrolysis, and that Cornelius O'Sullivan and Frederick William Tompson (1890) had shown that the activity of invertase in the presence of sucrose survives a temperature that completely destroys it if the sucrose is not present; they regarded this as an indication of the combination of enzyme and sucrose molecules.

Brown also made reference in his paper to the suggestion made by the great German organic chemist Emil Fischer (Fischer, 1894) that the specificity of enzyme action is to be explained in terms of the precise fitting together of enzyme and substrate molecules; this is referred to as Fischer's lock and key hypothesis. Normally a lock can only be operated by a given key; a slight modification to a key usually means that the key no longer works. It is somewhat ironic that although Fischer's ideas became particularly fruitful when

physical methods, such as kinetics and X-ray crystallography, were applied to them, he himself was scornful of such methods: Wilhelm Ostwald suggested to Fischer that physical methods would be helpful in organic chemistry, but Fischer replied bluntly "We have no use for your methods".

The next important step in enzyme kinetics was made by the German biochemist Leonor Michaelis and his Canadian assistant Maud Leonora Menten (Michaelis and Menten, 1913). They had observed that the effect noted by Brown — that the rate of reaction is independent of the concentration of the substrate — is only observed at higher concentrations of substrate. At lower concentrations the rate becomes proportional to the concentration of substrate. To explain this they considered the kinetic consequences of the idea that an enzyme–substrate complex is formed. The process can be represented as

$$E + S \rightleftharpoons ES \rightarrow E + products$$
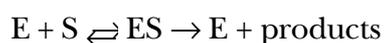
where E is the enzyme molecule, S the substrate molecule, and ES the complex. The idea of Michaelis and Menten was that an equilibrium is established between E, S and ES, and that the slow step is the breakdown of ES. Since usually there are many more molecules of substrate present than of enzyme (on account of the high molecular mass of the enzyme), the enzyme becomes saturated with substrate at higher substrate concentrations. The concentration of ES will therefore remain the same, and the rate will remain the same, as the concentration of substrate is varied. At low substrate concentrations, on the other hand, the enzyme will not be saturated, and the concentration of ES, and therefore the rate of reaction, will be proportional to the concentration of S.

This reasoning led to the well-known Michaelis–Menten equation, which contains a constant known as the Michaelis constant. This constant is defined in such a way that a high value means that there is only weak binding between the enzyme and the substrate; a low value means strong binding.

Some years later a more general formulation of the Michaelis–Menten equation was given by George Edward Briggs and the British geneticist John Burdon Sanderson Haldane (Briggs and Haldane, 1925). They pointed out that the Michaelis assumption that an equilibrium exists between E, S, and ES is not always justified, and should be replaced by the assumption that ES is present not necessarily at equilibrium but in a steady state. The resulting equation is of the same

form, but the Michaelis constant has a different significance with respect to the different rate constants.

Michaelis and his colleagues also made important contributions to our understanding of the way in which the rate of an enzyme-catalysed reaction is affected by the pH of the solution. For some time it had been noticed in many enzyme systems that the rate is low if the pH is high or low, and passes through a maximum at some intermediate value, which is usually not far from neutrality (pH 7). In 1911, two years before the formulation of the Michaelis–Menten equation, Michaelis and Davidsohn had concluded from this pH behaviour that the catalytic centres of enzymes must involve two ionizing groups (Michaelis and Davidsohn, 1911). For effective catalytic action one of these must be in the form in which it is capable of accepting a proton, while the other must be in a position to donate a proton. Over the years this basic idea was extended, particularly to take into account the ionization of the enzyme-substrate complex as well as that of the enzyme (Michaelis and Rothstein, 1920; von Euler, Josephson and Myrbäck, 1924; Waley, 1953; Laidler, 1955). A strong body of evidence shows that the powerful catalytic activity of enzymes is due in part to the fact that they function by being simultaneously able to donate a proton to, and accept another proton from, the substrate molecule. This has been referred to as a *push-pull mechanism.*

The basic idea that an enzyme reaction involves an enzyme–substrate complex as an intermediate is important, but requires much extension and elaboration. There is now a considerable body of evidence suggesting that in many cases there are two or more intermediates. A reaction scheme involving two intermediates may be written as

$$E + S \rightleftharpoons ES \rightleftharpoons ES' \rightarrow E + products$$

where ES´ is the second intermediate. Good evidence for such a mechanism has been obtained for the hydrolysis of acetylcholine by the enzyme acetylcholinesterase (Krupka and Laidler, 1961). In this system the rate goes through a maximum with increasing substrate concentration, and the evidence suggests that this is due to the attachment of substrate molecules to the second intermediate ES´.

With the purification and crystallization of proteins in the nineteen twenties, enzyme kinetics was able to enter a new phase. It became possible to study in much more detail the interactions between enzyme molecules and their substrates. This branch of enzyme

130

kinetics has been referred to as *molecular kinetics* (Butler, 1941). The frequency with which enzyme and substrate molecules react together is controlled by two main factors, the energy barrier to reaction, and the entropy of activation, which is the entropy change when the activated complex is formed from the reactants. Both of these factors provide valuable information about the molecular nature of the processes occurring. For example, a large positive entropy of activation is good evidence that charges are being neutralized when the process occurs; the entropy increase is due to the release of water molecules that had been bound by the ions. This is one example of a mechanistic detail that cannot easily be obtained from structural studies, since activated complexes do not exist long enough to be detected by any but the most sophisticated high-speed techniques.

The first to carry out kinetic studies with a pure enzyme, trypsin, was the British physical chemist John Alfred Valentine Butler (Butler, 1941). Many studies of the same kind have been made, with a variety of enzymes, and the results have contributed greatly to the understanding of enzyme action (Laidler and Bunting, 1973).

When an enzyme and a substrate are brought together, the steady state is usually established within a few milliseconds. As a matter of experimental convenience, most investigations of enzyme kinetics have been concerned with the steady state. To find out what is occurring while the steady state is being established — during what is called the transient phase of the reaction — requires special high-speed techniques. Two problems have to be overcome. The first is to bring the enzyme and substrate together rapidly (as otherwise the reaction may be over before they are properly mixed). The second is to make measurements within short periods of time.

The first problem may sometimes by overcome by the use of *flow methods,* in which solutions are forced together very rapidly. Suitable techniques were developed in particular by Roughton and his co-workers (Hartridge and Roughton, 1923; Millikan, 1936). An important variant of their method was the *stopped-flow method,* introduced by Britton Chance (Chance 1940) and later developed further (Gibson and Milnes, 1964). Sometimes the individual steps are too fast for their rates to be measured by flow methods, and then *relaxation methods* have to be used (Eigen, 1954ab). During more recent years much further work has been done using high-speed techniques, and many of the kinetic details of enzyme reactions have been worked out. The results have led to the realization that few such reactions appear to conform to the simple pattern first suggested by Michaelis and Menten; other reaction steps are usually involved.

REFERENCES

BROWN, A. J. (1892) "Influence of oxygen and concentration on alcohol fermentation" *J. Chem. Soc.* **61,** 369–385.

BROWN, A. J. (1902) "Enzyme action" *J. Chem. Soc.* **81,** 373–386.

BUTLER, J. A. V. (1941) "Molecular kinetics of trypsin action" *J. Amer. Chem. Soc.* **63,** 2971–2974.

CHANCE, B. (1940) "The accelerated flow method for rapid reactions" *J. Franklin Inst.* **229,** 455–478, 613–640, 737–766

EIGEN, M. (1954a) "Über die Kinetik sehr schnell verlaufender Ionenreaktionen in wässerige Lösung" *Z. physikal. Chem.* [N. F.], 1, 176–200.

EIGEN, M. (1954b) "Methods for investigation of ionic reactions in aqueous solutions with half lives as short as 10^{-9} s" *Disc. Faraday Soc.* **17,** 194–205.

FISCHER, E. (1894) "Einfluss der Configuration auf die Wirkung den Enzyme" *Ber. Dtsch. Chem. Ges.* **27,** 2985–2993.

GIBSON, Q. H. and MILNES, L. (1964) "Apparatus for rapid and sensitive spectrophotometry" *Biochem. J.* **91,** 161–171.

HARTRIDGE, H. and ROUGHTON, F. J. W. (1923) "A method of measuring the velocity of very rapid chemical reactions" *Proc. Roy. Soc. A,* **104,** 376–384.

KRUPKA, R. M. and LAIDLER, K. J. (1961) "Molecular mechanisms for hydrolytic enzyme action. Part II, Inhibition of acetylcholinesterase by excess substrate" *J. Amer. Chem. Soc.* **83,** 1448–1454.

LAIDLER, K. J. (1955) "The influence of pH on the rates of enzyme reactions. Part I. General theory" *Trans. Faraday Soc.* **51,** 528–560.

LAIDLER, K. J. and BUNTING, P. S. (1973) *The Chemical Kinetics of Enzyme Action,* Clarendon Press, Oxford, 2nd Edition, Chapter 7.

MICHAELIS, L. and DAVIDSOHN, H. (1911) "The theory of the isoelectric effect" *Biochem. Z.* **30,** 143–150.

MICHAELIS, L. and MENTEN, M. L. (1913) "Kinetik der Invertinwirkung" *Biochem. Z.* **49,** 333–369.

MICHAELIS, L. and ROTHSTEIN, M. (1920) "Theory of invertase action" *Biochem. Z.* **110,** 217–233.

MILLIKAN, G. A. (1936) "Photometric methods of measuring the velocity of rapid reactions. III. A portable micro-apparatus applicable to an extended range of reactions" *Proc. Roy. Soc. A,* **155,** 277–292.

O'SULLIVAN, C. and TOMPSON, F. W. (1890) "Invertase: a contribution

to the history of an enzyme or unorganized ferment" *J. Chem. Soc.* **57,** 834–931.

VON EULER, H., JOSEPHSON, K. and MYRBÄCK, K. (1924) "Computation of the acidity–pH curve of sucrase" *Z. physiol. Chem.* **134,** 39–49.

WALEY, S. G. (1953) "The chemistry of enzymic reactions" *Biochim. Biophys. Acta* **10,** 27–34.

WURTZ, C. A. (1880) "Sur la papaïne: contribution à l'histoire des ferments solubles" *Compt. rend.* **91,** 787–791.