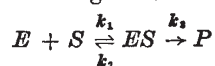


PHYSICAL CHEMISTRY OF ENZYMES

FARADAY SOCIETY DISCUSSION

THE Faraday Society held a discussion on "The Physical Chemistry of Enzymes" during August 10-12 in the Department of Physiology, University of Oxford. At the suggestion of the chairman of the organizing committee, Prof. F. J. W. Roughton, the time of the meeting was arranged so that it followed the International Congress in Biochemistry. As a result, a large number of biochemists from the Congress were able to be present at the Faraday Society discussion, and the total attendance was 244. Twenty-nine papers concerning a wide field of enzyme topics were discussed, and a great many valuable contacts were made between enzyme biochemists and physical chemists interested in the enzyme field. The chair at the opening session was taken by the president of the Society, Prof. R. G. W. Norrish, and the introductory paper was given by Dr. M. Dixon, whose lucid exposition of the present position of the field set the pattern for the subsequent discussion.

Dr. Dixon pointed out that of approximately four hundred and fifty known enzymes, all of them protein molecules, about a hundred have been obtained in crystalline form. He noted that enzyme studies include both protein properties and catalytic properties, and that most of the papers before the meeting were concerned with the latter aspect. A great many enzyme reactions fall into the general class of group transfer reactions, and the requirements of specificity suggest in many cases a close fit between enzyme and substrate over a patch 15-20 Å. in diameter. Such an active patch might be associated with a particular folding of polypeptide chains in the surface of the protein, giving rise to a characteristic arrangement of side-chains. Passing to problems of kinetics, Dr. Dixon discussed the various developments of the basic Michaelis-Menten equation, which supposes enzyme (E) and substrate (S) to combine to form a complex (ES) which decomposes to the product (P), with the rate-constants given:



He referred to the various ways of identifying the rate-determining step for the overall enzyme reaction, later treated in detail by several contributors to the discussion. He showed how the effect of pH on enzyme velocity may lead to a knowledge of the pK of ionizing groups in the active site, and also described the use of isotopes in investigating the sequence of bonds broken in enzyme reactions.

Characterization and Physical Properties

The papers in the section on "Characterization and Physical Properties" were concerned entirely with soluble enzymes. It is well known that a crystalline protein is not necessarily homogeneous. In cases where two or three species are present, they may frequently be separated by electrophoresis or sedimentation, the first technique depending on differences in charge (therefore ionizing groups) and the second on molecular weight. A single homogeneous protein diffusing across a boundary according to Fick's law sets up a concentration gradient/distance relationship of Gaussian type, but a mixture of proteins leads to departures from this curve. A paper by R. L. Baldwin, L. J. Gosting, J. W. Williams

and R. A. Alberty (Wisconsin) described first how the diffusion method has been increased in sensitivity until 0.1 per cent impurity is detectable in crystalline albumins, using an interference-fringe method. A study of the reversible spreading of electrophoretic boundaries can detect differences of one electronic charge between protein molecules.

V. Massey, W. F. Harrington and B. S. Hartley (Cambridge) have 'labelled' enzymes with an organic dye molecule, and compared the inhibiting effect of the dye with measurements of fluorescent depolarization and sedimentation constant. The evidence is that the first dye molecules taken up combine with an active group of the enzyme, and that the same group is involved in chymotrypsinogen, chymotrypsin and di-isopropylphosphofluoridate-chymotrypsin. The group concerned is therefore probably the enzyme-substrate-binding group, and it is of great interest that the group is free in the precursor molecule. Since the di-isopropylphosphofluoridate site is also essential for catalytic activity, this is associated with activation of the enzyme-substrate complex:



According to H. Neurath and W. J. Dreyer (Seattle), the conversion of the precursor chymotrypsinogen into the active enzyme chymotrypsin involves the hydrolysis of a peptide bond between arginine and an isoleucine residue, a similar process, but involving lysine, occurring in the trypsinogen-to-trypsin change.

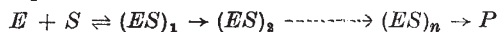
In the living cell the enzymes are in contact with lipoidal membranes which may modify their activity, and this aspect was considered by M. J. Fraser, J. G. Kaplan and J. H. Schulman (Cambridge), who found negatively charged oil-water emulsions to adsorb positively charged catalase very strongly, the first eight monolayers of catalase being completely inactivated. A positively charged oil droplet, however, only adsorbed one monolayer of enzyme, which still retained some activity. Liberation of catalase from living cells has been found to give an eighteen-fold increase in activity in agreement with the emulsion findings. A paper by L. Robert and J. Polonovski (Paris) dealt with the liberation of xanthine oxidase from the fat droplets in milk by various methods. If enzyme activity can be modified by adsorption on membranes, then a new dimension is added to enzyme biochemistry within the cell.

Kinetics and Mechanism

The second half of the meeting was devoted to a consideration of "Kinetics and Mechanism". A paper by J. G. Kirkwood (Yale) advanced the view that, in addition to the usual dispersion and ion-dipole forces, the fluctuation of proton configuration on the protein part of an enzyme will lead to an attractive electrostatic force on a substrate molecule at its surface. If now in the process of forming the activated complex ($ES \rightarrow ES^*$) there is an increase in dipole, then there will be a lowering of ΔG^* , the free energy change of activation, that is, a catalytic action on the substrate. The discussion turned on how far this mechanism will predict the details of the activity/ pH curves observed in practice—it accounts for the maximum observed in terms of only one arbitrary constant. A paper by K. J. Laidler (Washington,

D.C.) gave criteria for deciding the relative magnitudes of k_1 , k_2 and k_3 , and then tabulated ΔS^* (entropy of activation) in the various cases. Laidler stressed an interpretation of entropy changes in terms of water-release effects following polarity changes in the activation process.

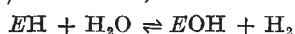
E. L. Smith, B. J. Finkle and A. Stockell (Utah) discussed the hydrolytic action of purified papain. They found the Michaelis constant K_M ($K_M = k_3 + k_2/k_1$) and k_3 to vary with pH in a rather complex manner, pH optima depending upon temperature. The explanation given was that k_3 involves a sequence of steps,



and that the rate-determining step changes with temperature. The papain molecule contains an SH-group in the active site which may function by forming a thiol ester with the carbonyl group of the benzoyl arginine amide substrate. R. Lumry and E. L. Smith (Utah) prefer to consider the Michaelis equation simply as the general equation for the conservation of enzyme molecules, replacing K_M by β/α and k_3 by β , and then seeking correlations between α and β for the carboxypeptidase system for a range of variables, pH , solvent, etc. These authors stressed the need for a proper statistical treatment of the experimental results.

Four papers were presented on esterases. J. A. Cohen (Netherlands) has worked with acetyl cholinesterase and chymotrypsin and confirmed the studies of Shaffer and Summerson, using di-isopropylphosphoridate labelled with phosphorus-32. Reaction of the enzyme with this substance and subsequent hydrolysis yields phosphoserine; but it seems unlikely that serine is the initial site of attack, as pK evidence points to histidine as the active site. It may be that the di-isopropylphospho-groups migrate from an initial histidine site to serine, and B. J. Jandorf *et al.* (Maryland) presented evidence that ageing chymotrypsin-di-isopropylphosphoridate makes it more and more resistant to reactivation by nucleophilic agents. I. B. Wilson (Columbia), in his paper, pictured the active site of acetyl cholinesterase as an anionic site next to an esteratic site. While nucleophilic agents are normally used for removing the di-isopropylphospho-group from the esteratic site, he found that incorporation of a cationic group in the agent much increased its speed of action. Presumably the cationic group is adsorbed on the anionic (enzyme) site and helps the nucleophilic attack. Bergmann compared monoquaternary and diquaternary inhibitors with acetylcholinesterase and butyrylcholinesterase, and on this basis concluded that the former contains two anionic sites, the latter one, and ordinary liver esterase none.

Two papers were contributed on bacterial hydrogenase, a particulate enzyme. That by A. Couper, D. D. Eley and A. Hayward (Bristol and Nottingham) found the pH_2 conversion to be about five times faster than either $D_2 - H_2O$ or hydrogenation reactions. The true activation energy for the conversion was considered to be 10 k.cal., and a dehydrated film of bacteria was inactive but became active again on rehydration. A. I. Krasna and D. Rittenberg (Columbia) have found that, in the H_2/D_2O exchange, HD was the first species to appear in the gas phase, and they put forward a scheme for the conversion involving interaction of an enzyme hydride (EH) with water,



D. E. Koshland (Brookhaven) showed how isotope exchange may be used for excluding possible mechanisms of action of transferring enzymes, illustrating his methods with experiments on 5^1 -nucleotidase. In this case he was able to exclude an enzyme-phosphate complex as an intermediate in the hydrolysis of adenosine monophosphate. It would seem that both adenosine monophosphate and water must be adsorbed on the enzyme, and also that the adenosine serves to fix the orientation of active groups. R. K. Morton (Melbourne) has investigated the transferring activity of phosphatases with acceptors such as glucose, finding a competition between transferring and hydrolysis reactions, and from this concluding that there is a definite water-site in the enzyme.

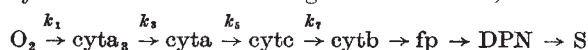
G. Weber (Sheffield), in a theoretical paper, considered the transfer of energy between separated enzyme and substrate molecules, and from the protein part of an enzyme-substrate complex into the substrate molecule. He concludes that the latter is probably the way that the enzyme-substrate activated complex is formed. A theoretical paper by A. G. Ogston (Oxford) considered equilibria between enzyme, enzyme-substrate, enzyme-inhibitor and enzyme-substrate-inhibitor complexes and then discussed the free energy of activation ΔG^* for various limiting conditions. H. Gutfreund (Cambridge) outlined the results of applying the analysis of Gutfreund and F. J. W. Roughton to the initial non-stationary state of several hydrolytic enzymes, using a Gibson stopped-flow apparatus. This method leads to values of k_1 for formation of the enzyme-substrate complex, and a later paper by E. C. Slater (Amsterdam) showed how conventional kinetic data may also lead to values of k_1 . Generally, k_3 is not simply a monomolecular decomposition, but is really k_3^1X , where X is water or some other acceptor. By examining a given enzyme with a range of acceptors, a relation between apparent K_m and V_{max} ($= k_3e$) is obtainable, which leads to values of k_1e and k_2e , where e is the enzyme concentration.

Sir Rudolph Peters (Agricultural Research Council) compared the toxic properties of the natural fluorocitric acid with the synthetic material, containing unnatural isomers. The synthetic material is the more toxic with soluble aconitase, and less toxic with the mitochondrial preparation, which Sir Rudolph tentatively attributed to stereochemical differences between the preparations. Stereochemistry was a main consideration in the paper by B. Vennesland (Chicago). She has found that deuterated diphosphopyridine nucleotide is stereospecific when formed enzymatically, but not when formed by chemical reduction, showing that in the enzyme case the deuterium atoms must add on to the same side of the pyridine ring.

A further two papers dealt with enzymatic reductions by the reduced diphosphopyridine nucleotide. H. Theorell (Stockholm) examined the kinetics of reduction of aldehyde using liver alcohol dehydrogenase. He has established that the reduction goes through the binary complex of reduced diphosphopyridine nucleotide and liver alcohol dehydrogenase and has obtained values for the four rate-constants concerned. In the case of yeast alcohol dehydrogenase, it was mentioned that a ternary complex is involved. I. W. Sizer and A. Gierer have started from Westheimer's proof, using deuterium labelling, that in the transfer from reduced diphosphopyridine nucleotide to acetaldehyde the hydrogen atom goes to the carbon of the acetaldehyde. Arguing from the observed effects of pH on velocity and K_m , they

conclude that the second hydrogen atom is provided by the enzyme and not by an H_3O^+ ion from the solution.

The last three papers at the meeting were concerned with iron porphyrin systems. P. George and G. I. H. Hanania (Cambridge) discussed titration data for myoglobin derivatives, concluding that only one haem-limited acid group is present. Q. H. Gibson (Sheffield) and F. J. W. Roughton (Cambridge) described work on evaluating velocity constants for the addition of four carbon monoxide molecules successively to the four iron atoms of the haemoglobin molecule, and similar equilibrium constants with oxygen. The solutions for the equations were obtained using electronic computation. In the absence of differences in reactivity, the velocity constants should be in the ratios 4, 3, 2, 1, where 1 refers to addition of the fourth molecule. In fact, the first three constants do not vary much from this expectation, but the fourth constant is enhanced eighty-fold. Gibson described the experimental method used as an adaptation of the flash photolysis technique of Norrish and Porter. B. Chance (Pennsylvania) described work on determining pseudo first-order velocity constants for the steps of the cytochrome chain occurring within the cell,



where fp denotes flavoprotein. Using different wavelengths, it was possible to follow the concentrations of the individual components by spectrophotometric means, and Chance concludes that there is a graded series of reaction-rates from left to right along the chain. This fact must be taken into account in any proposed mechanisms for electron transport. Work was also described with nitrate as oxidizing agent and with photosynthetic systems.

It has only been possible to mention points from each paper in the above brief account, and reference to the lively discussions which followed the papers has had to be omitted. The Faraday Society is to be congratulated on its foresight in bringing together biochemists and physical chemists in what has proved to be one of the most stimulating discussions in recent years.

D. D. ELEY

PULMONARY CIRCULATION AND RESPIRATORY FUNCTION

SYMPOSIUM IN DUNDEE

DURING September 15-16, Queen's College (University of St. Andrews), Dundee, organized a symposium on "Pulmonary Circulation and Respiratory Function", which was probably the first of its kind to be organized by a university institution. It was an interesting experiment because the papers read covered a wide range of subjects and the audience was drawn from a wide range of interests—namely, physicians and surgeons, pathologists, physiologists and applied physiologists. The interest taken in the symposium, to judge from the size and enthusiasm of the audience, was in large part due to the development during recent years of human cardio-respiratory studies all over the world. Much of the work discussed has not yet been adequately assessed from the point of view either of its scientific reliability, or its value in assisting in diagnosis.

Dr. de Burgh Daly (Institute of Animal Physiology, Cambridge) opened the programme with a paper on "Pulmonary Vasomotor Nerve Activity and its Possible Functional Significance". He pointed out that a great deal of careful experimental work on animals is needed to demonstrate the existence of changes in pulmonary vascular resistance due to the action of vasomotor nerves. Valid conclusions can only be reached from the results of animal experiments where factors such as the pulmonary ventilation and circulation can be controlled adequately. Without these precautions, passive changes in pulmonary flow, pressure and resistance may obscure the demonstration of active vasomotor changes. The demonstration of vasomotor responses, as Dr. Daly remarked, does not imply that they play any part in the responses of the intact animal. The conditions of these experiments are often highly artificial. In the human subject, the control of all the variables is so difficult a task that great care should be exercised in drawing any conclusions about the existence of an independent control of the pulmonary blood vessels. Even in animal experiments, the site of action of pulmonary vasomotor fibres is uncertain.

Dr. K. W. Donald (Department of Medicine, Birmingham) gave a paper on "The Pulmonary Circulation in Health and Disease", from which one gathered that the difficulties in designing appropriate experiments, to which Dr. Daly directed attention, must apply also to experiments on man. For example, though there is agreement that the pulmonary vascular resistance to blood flow is small, the effect of exercise on the resistance of the blood vessels is in dispute even in healthy subjects. Some workers consider that the resistance decreases during exercise, whereas others find that the pulmonary vascular bed acts passively in conducting blood. On this matter, there appears to be no agreement about the facts, so it is superfluous to consider whether any question of vasomotion is involved. Dr. Donald concluded that there is as yet no clear evidence of vasomotor activity in human lungs. The problems of physiology posed by disease are numerous and largely unsolved, and some of these were reviewed.

Prof. A. A. Liebow (Yale) stressed the importance of the bronchial arteries, the azygos and other systemic veins in the circulation. His experiments showed that it is possible to ligate the pulmonary arteries and veins and yet have a collateral circulation of oxygenated blood (the extent of which increased in the course of a few months) adequate to maintain life. He demonstrated these points with the aid of casts of the blood vessels and suggested that his experiments may have some application in the treatment of certain developmental abnormalities of the heart and large vessels, and in coronary occlusion. Sir Russell Brock (Guy's Hospital, London) directed attention to the studies which have been made on the physiology of the infundibulum of the right ventricle. Clinical observation both before and at operation in cases of Fallot's tetralogy, for example, make it essential that detailed information should be available about the conditions existing in the infundibulum before a successful operative result can reasonably be expected. He illustrated his thesis by reference to a number of clinical cases.

Prof. J. Gough (Welsh National School of Medicine, Cardiff) reported on the results of post-mortem examinations made in his Department on Welsh coal miners. There is a high incidence of deaths attributable to cor pulmonale and massive pneumoconiosis.