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OF
ENZYME ACTION
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Edited by

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OPENING REMARKS

THE great advance which has recently taken place in enzymology is well shown by the fact that it is possible to hold such a symposium as this, on the molecular basis of enzyme action. Only a short time ago such a discussion would have been mainly a matter of supposition and guesswork, but to-day it can be firmly based on a foundation of definite knowledge about the active centres of enzymes, in relation both to their mode of action and to their structure.

By way of introduction to the symposium, it may be of some interest to recall that the change has been brought about by the development of several powerful methods by which the problem can be attacked from different angles.

There is first the recent great development of enzyme kinetics, which makes it possible in many cases to analyse the complete reaction into a sequence of steps. Then the study of specificity and of competitive inhibition has revealed much about the manner in which enzyme and substrate combine. The application of isotopic methods to enzymes has thrown light on the nature of the reaction in many cases, and the study of enzymatic catalysis of isotopic exchange reactions is a very powerful tool, permitting the study of partial reactions, in particular of the formation of intermediate enzyme complexes apart from their breakdown, often revealing their nature. In certain cases reactive intermediates may be detected by special physical methods, especially by spectroscopic or fluorimetric methods or by one of the resonance techniques. A further interesting method is by the study of artificial models which imitate the action of enzymes. All these methods relate to the mode of action of the active centre.

But there have also been great advances in the knowledge of the structure of active centres. On the one hand, a number of special prosthetic groups have been identified chemically: metal atoms, haems, flavins, pyridoxal phosphate, biotin and others. On the other hand, the actual amino-acid sequences of the peptide chains in active centres are now being elucidated by recently developed methods. Even where it is not possible to determine the sequence of the whole protein, the fact that active centres contain unusually reactive groups may permit a specific labelling of the

centre with radioactive reagents, followed by the determination of the sequence in the neighbourhood of the label. However, this is not enough; the active centre may extend over more than one peptide chain, and in order to determine its complete structure the folding of the chains must also be determined. This has yet to be done.

The subject is now at a very interesting stage, when knowledge of the structure is being correlated with knowledge of the mode of action of enzymes; this is the main theme of the symposium, which will include many examples of the various methods of attack which I have mentioned.

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ENZYMES AND ENZYME MODELS

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THE determination of mechanisms of enzyme action is among the great problems of modern chemistry.[1, 2, 3] Although not one enzymatic process is today understood, and although no model system has achieved catalytic activity comparable to that of the corresponding enzyme, considerable progress has been made in elucidating the "active sites" in enzymes, and in finding catalytic systems which will operate (although slowly) under experimental conditions which are comparable to those in cells and, hopefully, by mechanisms which parallel the biochemical ones. The problem is under attack from many angles; studies of peptide sequences, X-ray analysis of protein structure, determination of the coenzyme or active sites needed for reaction, kinetic studies of enzyme action, and chemical "models" for various enzymatic systems. All of these methods provide information relevant to the central problem: how does a protein strongly accelerate a chemical reaction between a substrate and either a coenzyme molecule or an active site on the protein itself or some other reagent.

In subsequent sections of this review, some individual enzymes are considered in some detail. Before considering these systems, a few words might help set the problem in perspective. Much of our understanding of catalytic action has been gained by studies of model systems. The pioneers in such investigations[4, 5] did not have the present-day background of theoretical chemistry on which to build. In 1947, in a paper pertinent to modern consideration of mechanism, Braunstein[6] suggested the outlines of a mechanism for transamination; this mechanism (somewhat modified)[7] and the role of pyridoxal have been substantiated[7,8] and are generally accepted today. More recently, the role of thiamin in the decarboxylation of pyruvate,[9] the action of diphosphopyridine nucleotides in the direct and stereospecific transfer of hydrogen,[10] and the role of the serine residue in the reactions of esterases[11, 12, 13] and of phosphoglucomutase[14] have been discovered, and imitated with model systems. Some of these systems

are here considered. The role of biotin and of several other coenzymes in enzymatic processes will be discussed in another session in this Congress. In a recent summary,[3] the author stated, "...hopefully, in another decade, reviews may be concerned with enzymes and enzyme analogs (rather than with models)...". The decade has not yet passed, and the prediction still seems reasonable.

RIBONUCLEASE

Structure

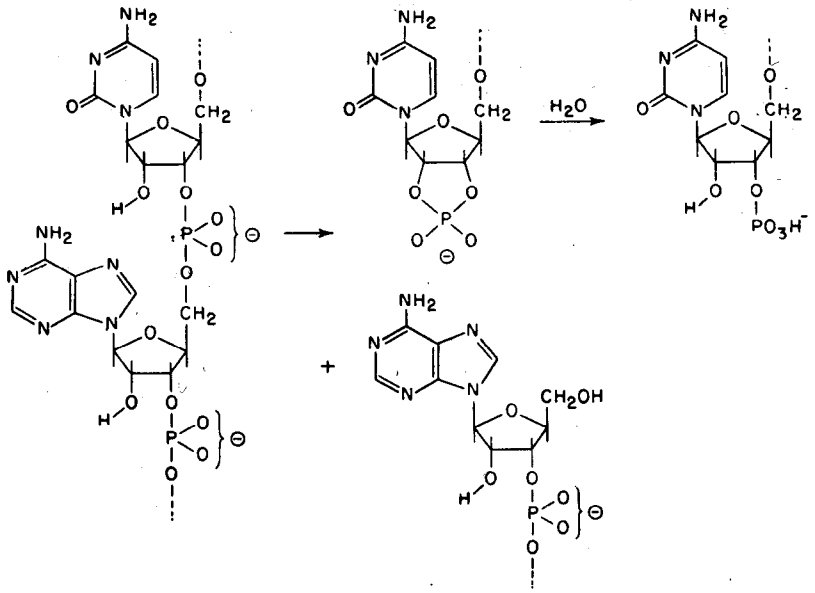
Ribonuclease is distinguished as the first enzyme for which the entire amino-acid sequence, and the location of the disulfide bonds is known. The signal achievement of determining this structure is largely the work of Moore *et al.*[15] at the Rockefeller Institute. Nevertheless, the mechanism of action of this enzyme is probably less well understood than that of others where structural information is less complete. Furthermore, although the sequence of amino-acids for this enzyme is known, the three-dimensional configuration may only be guessed. Neither the extent of α– helix nor the interactions of various helical and randomly coiled sections may be specified with assurance. The observed changes in optical rotation on opening the disulfide bonds suggest[16] that the net contribution of right-handed helix (i.e. the excess of right-over left-handed helix) is small, and probably only of the order of 20 per cent. (Although it is generally assumed that right-handed helices predominate in proteins, the balance must be a delicate one. In synthetic polypeptides, poly-L-benzyl glutamate forms a right-handed helix, but poly-L-benzyl aspartate a left-handed one.[17]) Alternate estimates of the helical content of ribonuclease, obtained from infra-red measurements and from the rapidity of hydrogen-deuterium exchange[16, 18] suggest a total of about 50 per cent of helical sections in the enzyme.

Chemical Intermediates

The enzyme catalyzes the cleavage of ribonucleic acid at the phosphorus to oxygen bond connecting the phosphate ester group of a pyrimidine nucleotide at 3′ to the 5′-position in the ribose residue of the adjacent nucleotide. The enzymatic reaction almost certainly proceeds in two steps;[19, 20] first, the formation of a 2′, 3′-cyclic nucleotide, with cleavage of the P–O bond to the 5′-position of the adjacent residue, and second, the opening of the cyclic phosphate.

Non-enzymatic Model Systems

The non-enzymatic hydrolysis of dialkyl phosphates takes place very slowly. For example, the hydrolysis of dimethyl phosphate has a half-time



of a day at 125° in 1 N alkali,[21] and a half-time of 5 hr at 100° in 5 M perchloric acid solution.[22] Furthermore, under both sets of experimental conditions, the hydrolysis occurs largely at the carbon to oxygen rather than at the phosphorus to oxygen bonds.[22] On the other hand, phosphate esters which hold an hydroxyl group adjacent to the P—O linkage hydrolyze comparatively readily[23] and presumably at the P—O bond. Considerable evidence shows that, in these instances, a five-membered cyclic phosphate is an intermediate; the reaction then consists of an internal displacement by the alcoholic group on phosphorus, which is highly favored over the corresponding external displacement by water or hydroxide ion.[24] The five-membered cyclic phosphate so formed is rapidly hydrolyzed: the opening of potassium ethylene phosphate in alkali[21] proceeds about 10^7 times as fast as does the hydrolysis of potassium dimethyl phosphate, and about 10^6 times as fast as does that of potassium trimethylene phosphate.[25] Similarly, the alkaline hydrolysis of methyl ethylene phosphate proceeds about 10^6 times as fast as does that of trimethyl phosphate, and about 1000 times as fast as does that of dimethyl hydroxyethyl phosphate.[26] Relative to the trialkyl ester, the hydroxyester hydrolyzes very rapidly, just as ribonucleic acid hydrolyzes more readily than does deoxyribonucleic acid. The high rates of the hydrolysis of the cyclic as compared to those of the non-cyclic esters invites speculation as to the origin of the large difference in rate. Presumably the proximate cause of the rapid reaction

is strain in the five-membered ring; the ultimate reason for this strain is still unknown.[27] This strain has, however, been measured thermochemically with methyl ethylene phosphate, and amounts to about 7–8 kcal per mole.[28] The comparative rates cited above suggest that the reactions catalyzed by ribonuclease are those which occur rather readily non-enzymatically.[29, 30]

How fast, then, is the enzymatic reaction relative to the corresponding non-enzymatic processes? The data on which a firm comparison could be made are not at hand. The second-order rate constant for the alkaline hydrolysis of potassium ethylene phosphate[21] is known and the reaction proceeds exclusively with P—O cleavage.[27] The rate of hydrolysis at pH 7 can be calculated on the assumption that the reaction rate is proportional to the hydroxide ion concentration at all pH, and that neither the components of buffers nor water effects the opening of the ring. Since water opens the ring in methyl ethylene phosphate,[26] the above assumption for potassium ethylene phosphate is unlikely to be valid. Nor are adequate kinetic data available for the enzyme. On the basis of the conviction that a crude estimate is better than none, the author suggests that, if the assumptions above were valid, the enzymatic rate of ring opening[20] for uridine-2, 3-phosphate would exceed the non-enzymatic one for ethylene phosphate by a factor of 10^9. For the reasons already cited, this figure probably considerably exaggerates the extent of the enzymatic catalysis; the catalysis is nevertheless real and of large magnitude.

Detailed Structural Information

Any detailed hypothesis for the action of ribonuclease must necessarily take into account many pieces of information and many deductions (some of which may prove misleading) which have been obtained concerning the enzyme. Richards[31] has cleaved ribonuclease with a special subtilisin into two parts, which he calls the S-protein and S-peptide; the latter consists of the twenty amino-acids from the N-terminal sequence of the original enzyme. Although neither the S-protein nor the S-peptide has enzymatic activity alone, the combination is fully active, and the equilibrium constant[20] for the dissociation of the peptide is probably less than 10^{-9} m/l. The S-peptide then has some (but not all) the properties of an artificial coenzyme; it is not chemically bonded to the protein but is held in place by the same sort of forces as those which bind DPN^+ to alcohol dehydrogenase or thiamin pyrophosphate to carboxylase.

The forces which hold the S-peptide to the S-protein have been investigated by Richards, who has chemically modified the S-peptide and then