Eagles, P. A. M., Johnson, L. N., Joynson, M. A., McMurray, C. H. & Gutfreund, H. (1969). *J. molec. Biol.* **45**, 533.
Morse, D. E. & Horecker, B. L. (1968). *Adv. Enzymol.* **31**, 125.

Imidazole and Proton Transfer in Catalysis

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The role of imidazole as a catalyst for group transfer by nucleophilic attack and proton transfer in chemical and enzymic reactions will be reviewed. There is strong evidence that a single imidazole group is involved in catalysis by some (but not all) 'serine' acylhydrolases, particularly chymotrypsin. The reasons for the special effectiveness of imidazole as a nucleophilic catalyst include its tertiary amine, cyclic structure and the possibility of resonance interaction between the imidazole ring and the carbonyl group in the transition state and product of nucleophilic attack; the acetylimidazole product is highly reactive because of the absence of normal amide resonance stabilization and the ease of protonation to the acetylimidazolium ion. Comparison with reactions of 1-acetyl-3-methylimidazolium ion demonstrates that most reactions of acetylimidazole with nucleophiles proceed through the *N*-protonated species. However, in spite of these attractive properties there is no firm evidence that imidazole is utilized by enzymes as a nucleophilic catalyst for acyl transfer; it is a nucleophilic catalyst for enzyme-catalysed phosphoryl transfer, but it does not show unusual reactivity as a non-enzymic catalyst for phosphoryl transfer.

Imidazole is also an effective catalyst for proton transfer in acyl transfer reactions, as shown most directly by its catalysis of hydrolysis and transfer reactions of acetylimidazole. Some progress towards understanding the detailed mechanism of this catalysis has been made by comparison with reactions of 1-acetyl-3-methylimidazolium ion catalysed by methylimidazole. The fact that catalysis of the hydrolysis of this compound is observed means that catalysis involves proton transfer to or from the carbonyl or water oxygen atoms. The absence of catalysis for the reaction with ammonia and the fact that catalysis does occur in the imidazole-catalysed reaction with acetyl-imidazole suggest that catalysis of the latter reaction involves proton donation by imidazolium ion to the leaving imidazole group (which is not possible in the acetylimidazolium ion reaction). The importance of proton transfer for these reactions is demonstrated by the very large sensitivity of the reactions of (unprotonated) acetylimidazole to the

basicity of the nucleophile and the fact that many reactions of acetylimidazole with nucleophiles are, in fact, general-base catalysis of hydrolysis rather than acyl transfer to the nucleophile. Solvent deuterium isotope effects and structure-reactivity correlations provide some evidence that imidazole acts as a catalyst for proton transfer in enzymic reactions. Teleologically it is not unreasonable that it should act as a general acid-base catalyst, rather than a nucleophilic catalyst, because its efficacy as a nucleophilic catalyst is low for normal substrates with poor leaving groups, whereas it is an effective general-base catalyst for such reactions.

The detailed mechanism of proton transfer in general acid-base catalysis of acyl- and carbonyl-group reactions is unknown. The proton transfer in most such reactions is in some sense 'concerted', but there are examples of apparent stepwise proton transfer, in which a diffusion-controlled proton-transfer step is rate-determining. Both mechanisms provide attractive methods for catalysis by enzymes, since enzymes cannot bring about local increases in H^+ or OH^- ion activity to facilitate required proton-transfer steps.

Imidazole Catalysis in Model Reactions for 'Serine' Peptidase and 'Cysteine' Peptidase Activities

By G. M. BLACKBURN, H. L. H. DODDS and D. J. SHIRE. (*Department of Chemistry, University of Sheffield, Sheffield S3 7HF, U.K.*)

Acyl-group transfer from *N*-acetyl-*O*-formyl-serine amide to glycine amide is catalysed by imidazole. The transition state for the reaction is closely related to that for hydrolysis of a glycine peptide by a serine peptidase, such as chymotrypsin, and the catalytic activity of imidazole therein can be assigned without ambiguity.

Imidazole accelerates the transfer of acyl groups from thiol esters to amines, such as glycine ester, by means of both general-acid and general-base catalysis at physiological pH values. These intermolecular reactions are more complicated than those involving oxygen esters, since some of the proton-transfer steps approach in rate that of a diffusion-controlled reaction (Barnett & Jencks, 1969) and may become rate-limiting. Nevertheless it is possible to assign catalytic roles to imidazole in the transition states for acyl-group transfer from sulphur to nitrogen. These provide a chemical analogy for the mechanism of thiolysis of amides by cysteine peptidases, in which the catalysis is basically different from that projected for serine peptidases. It will be shown that similar juxtaposition of functional groups in the active sites of enzymes, as shown by X-ray crystallography, need

not imply similar chemical roles for their catalytic function.

Barnett, R. E. & Jencks, W. P. (1969). *J. Am. chem. Soc.* **91**, 2358.

Catalysis by Carboxyl Groups

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The role played by intra-complex general-acid catalysis in the mechanism of action of glycosidases will be discussed and compared with intra- and inter-molecular general-acid catalysis in the hydrolysis of glycosides and acetals. The relative efficiencies of the carboxyl group and other acidic groups in the latter processes will be compared. The likelihood of intra-complex general-acid catalysis by groups other than the carboxyl group occurring in reactions catalysed by glycosidases will be discussed in the light of these results.

Possible factors that control the steric course of reactions catalysed by glycosidases will be discussed. An example of nucleophilic catalysis by a carboxylate group in the acid-catalysed hydrolysis of an acetal will be reported.

Steric Assistance for Intramolecular Catalysis

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Intramolecular catalysis by the carboxyl group of the hydrolysis of the amide or peptide linkage is a familiar but not fully understood reaction. Intramolecular reactions of monoesters derived from simple aliphatic dicarboxylic acids are accelerated by alkyl substitution on the intervening carbon chain: this effect has been explained in terms of a decrease in the number of unproductive conformations available to the more substituted isomer (Bruice & Pandit, 1960). But the similar effect of alkyl substitution in the reactions of the corresponding amides is observed also for compounds derived from maleic acid. The hydrolysis of a half-amide of methylmaleic acid is some 30 times as fast as that of the maleic acid derivative, and the hydrolysis of the half-amides of dimethylmaleic acid is accelerated by an even larger factor. The reaction involves rapid cyclization to form the anhydride, and dimethylmaleic anhydride is actually less reactive than the starting amide. [The reaction has been used by Dixon & Perham (1968) for the reversible blocking of protein amino groups.]

This promotion of the rates of intramolecular reactions that are already very fast seems to be a direct result of relatively small changes in molecular geometry, and this work is intended to clarify the

factors involved. If the rate enhancement due to intramolecular catalysis can be increased, as here, from about 10^5 to 10^9 simply by replacing two hydrogen atoms by methyl groups, then similar effects are surely to be expected in enzyme-catalysed reactions.

Bruice, T. C. & Pandit, U. K. (1960). *J. Am. chem. Soc.* **82**, 5858.

Dixon, H. B. F. & Perham, R. N. (1968). *Biochem. J.* **109**, 312.

Amino and Aromatic Groups in Enzymic Catalysis: Active and Allosteric Sites of Glutamate Dehydrogenase

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In the study of the mechanism of enzyme action, attention has increasingly been focused on the clarification of the chemical and biological role of some functional groups, located in specific regions of the protein molecule, which are either directly involved in the formation of the enzyme-ligand complex, or play a more indirect role in the mechanism of action. The amino acid residues bearing such groups have been termed 'contact' and 'auxiliary' residues respectively (Koshland, 1960). For the understanding of the role of these residues in enzymic catalysis their identification is only the first step: detailed study of their chemical reactivity then provides auxiliary and helpful information.

Chemical modification of proteins has therefore become a widely used tool for elucidation of the mechanism of enzyme action. As a result of these modifications, stable covalent bonds between the protein and the modifying agent are usually established; occasionally intermediate complexes may form. Thus labelling by substrate, by quasi-substrate and by non-specific reagents and affinity labelling (Koshland, 1960; Singer, 1967) have been used successfully with several enzymes.

Among the amino acid residues that may occur at or near the enzyme binding sites, lysyl, tyrosyl and tryptophyl residues have been detected in several enzymes. All show rather useful properties: for instance, they are relatively easy to label with suitable inorganic or organic reagents.

Our discussion will be centred mainly on some chemical reactions of amino and aromatic groups. These reactions (acetylation, guanidination, dinitrophenylation, amidination, iodination, nitration and reaction with *N*-acetylimidazole, with *N*-bromosuccinimide etc.) are all widely used at present in enzyme protein studies. Perturbation effects and fluorescence characteristics, both related to aromatic structures present in the protein molecule, will also be considered. Several difficulties