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

By B. Capon. (*Department of Chemistry, University of Glasgow, Glasgow W.2, U.K.*)

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

By A. J. Kirby and P. W. Lancaster. (*University Chemical Laboratory, University of Cambridge, Cambridge CB2 1EW, U.K.*)

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

By G. di Prisco, B. D'Udine, M. Pepe and F. Scalenghe. (*International Laboratory of Genetics and Biophysics, Naples, Italy*)

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

must be taken into account during examination of these reactions, since, besides the general danger of denaturation, the complexity of a protein molecule, where many reacting groups form a unique environment, may bring about considerable alterations in the chemical reactivity of these groups, which often does not closely resemble that shown by the same groups in simpler compounds.

Ox liver glutamate dehydrogenase is one enzyme that has recently been the object of chemical modifications. Although these investigations are still at a very preliminary stage, the studies on the active and regulatory sites of this enzyme that we and other investigators are carrying out indicate that both amino and aromatic groups are involved in the reactivity of the enzyme sites. Fisher & Cross (1965) have suggested, on the basis of solvent-perturbation difference spectra, that tryptophan is present at the glutamate-binding site, and these findings are consistent with those of Brocklehurst *et al.* (1970). Colman & Frieden (1966*a*,*b*) have shown that enzymic activity is lost and binding constants of allosteric modifiers are altered by acetylation of several lysyl residues. The use of dinitrophenylation (di Prisco, 1967) has led to the finding that this enzyme possesses two separate or perhaps partially overlapping regulatory sites, one for GTP and the other for ADP. The reactivity of the regulatory sites, as well as that of the catalytic ones, which can be selectively protected by the presence of the specific ligand, is lost when, in each site, a set of six lysyl and three tyrosyl residues per molecule of active oligomer, composed of six, probably identical (Appella & Tomkins, 1966), polypeptide chains of molecular weight 53000 (Eisenberg & Tomkins, 1968) has been dinitrophenylated (G. di Prisco, unpublished work). In agreement with these findings, Anderson, Anderson & Churchich (1966) have shown inhibition by pyridoxal phosphate, which, after reduction and total hydrolysis, was found combined in a Schiff base form with a lysyl residue involved in the reactivity of the catalytic site. The essential role of lysyl and tyrosyl residues in the catalytic and allosteric properties of the enzyme has been confirmed by different techniques, involving reaction with some other group-specific reagents (Brocklehurst *et al.* 1970).

Anderson, B. M., Anderson, C. D. & Churchich, J. E. (1966). *Biochemistry, Easton*, **5**, 2893.

Appella, E. & Tomkins, G. M. (1966). *J. molec. Biol.* **18**, 77.

Brocklehurst, J. R., Dodd, S. H., Freedman, R. B., Malcolm, A. D. B., Price, N. C. & Radda, G. K. (1969). In *Advanced Study Institute on Pyridine-Dependent Dehydrogenases, Konstanz.* Ed. by Sund, H. Berlin: Springer-Verlag.

Colman, R. F. & Frieden, C. (1966*a*). *J. biol. Chem.* **241**, 3652.

Colman, R. F. & Frieden, C. (1966*b*). *J. biol. Chem.* **241**, 3661.

di Prisco, G. (1967). *Biochem. biophys. Res. Commun.* **26**, 148.

Eisenberg, H. & Tomkins, G. M. (1968). *J. molec. Biol.* **31**, 37.

Fisher, H. F. & Cross, D. C. (1965). *Biochem. biophys. Res. Commun.* **20**, 120.

Koshland, D. E., jun. (1960). *Adv. Enzymol.* **22**, 45.

Singer, S. J. (1967). *Adv. Protein Chem.* **22**, 1.

The Reactivity of Group-Specific Chromophoric Reagents

By D. J. Birkett, R. B. Freedman, N. C. Price and G. K. Radda. (*Department of Biochemistry, University of Oxford, Oxford OX1 3QU, U.K.*)

The chemical modification of reactive side chains in enzymes can be used to obtain information about conformation, 'essential' groups and the role of such side chains in subunit interactions. In addition, if the reagent has environmentally sensitive optical properties, it can be used to explore the nature of specific sites.

Two group-specific reagents will be described.

2,4,6-Trinitrobenzenesulphonic acid has been used to characterize the reactivities of amino groups in several enzymes (Freedman & Radda, 1969). When the extent of reaction is compared with changes in activity and allosteric response, particular amino groups can be associated with these enzymic functions. 2,4,6-Trinitrobenzenesulphonic acid can also be employed to detect conformational changes (e.g. between the oxidized and reduced forms of cytochrome *c*).

7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole (Ghosh & Whitehouse, 1968) reacts rapidly with thiol groups to give a fluorescent product. The uses of this reagent for the study of glyceraldehyde 3-phosphate dehydrogenase and muscle phosphorylase will be discussed.

Freedman, R. B. & Radda, G. K. (1969). *Biochem. J.* **114**, 611.

Ghosh, P. B. & Whitehouse, M. W. (1968). *Biochem. J.* **108**, 155.

X-Ray Analysis of an Unusual Reaction in Lysozyme

By C. C. F. Blake. (*Department of Zoology, Laboratory of Molecular Biophysics, University of Oxford, Oxford OX1 3PS, U.K.*)

The iodination of lysozyme in acid media results in a product that is enzymically inactive. The reaction has been carried out in the crystalline state. X-ray analysis of the product by high-resolution difference Fourier methods show that glutamate-35 and tryptophan-108 have undergone an unusual reaction caused by environmental factors. The loss