PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 501st Meeting of the Society was held in association with the Chemical Society and with the Molecular Enzymology and Proteins Groups of the two Societies, at Oxford on Tuesday and Wednesday, 6 and 7 January 1970, when the following papers were presented:

SYMPOSIUM ON 'CHEMICAL REACTIVITY AND BIOLOGICAL ROLE OF FUNCTIONAL GROUPS IN ENZYMES'

Functional Thiol Groups in Enzymic Catalysis

By F. LYNEN. (*Max-Planck-Institut für Zellchemie, Munich 2, Germany*)

Recent advances in the elucidation of the three-dimensional structures of some enzyme molecules support the idea that enzyme catalysis can be described completely in terms of organic chemistry. The reaction between enzyme and substrate has a number of consequences.

(a) Substrates of a bimolecular reaction are brought in close proximity on the enzyme surface.

(b) The substrate molecules are fixed on the enzyme in a particular conformation that may facilitate chemical reactions.

(c) The atoms of the substrate come in close contact with amino acid side chains or other functional groups present at the active site of the enzyme. These groups may take an active part in enzyme catalysis by directing the process over energetically more favourable intermediates.

(d) The amino acid side chains in the active site may form a microenvironment that can dramatically affect the stabilities of transition states, and hence reaction rates.

The special role of thiol groups in enzymic catalysis is related to their ability to form thiol esters with carboxylic acids. In thiol esters the carbonyl function is particularly pronounced because the electrons of the C-S bond are polarized in the direction of sulphur (Jaenicke & Lynen, 1960). As a result transfer reactions of acyl derivatives of thiol compounds [derivatives of bound cysteine, cysteamine (as in CoA and the acyl-carrier protein), or reduced lipoic acid] involve a nucleophilic attack of the acceptor molecules on the positively charged carbon atom of the polarized carbonyl group. The reactions of thiol esters also include condensation reactions at the α -carbon of the acyl group exemplified by: (a) the ester condensation with acyl thiols to form thiol esters of β -oxo acids; (b) aldol condensation with oxo compounds; (c) carboxylation by *N*-carboxybiotin.

Eggerer (1965) demonstrated the formation of enolic acetyl-CoA by isotopic experiments with citrate synthase as catalyst. The uptake of ^3H from

$^3\text{H}_2\text{O}$ into the methyl group at physiological pH was dependent on the presence of (*S*)-malate as an inducer. It was postulated that (*S*)-malate is bound by the enzyme in a fashion analogous to the binding of oxaloacetate in the physiological reaction. As a result one of its carboxyl anions comes in close contact with the methyl group of the enzyme-bound acetyl-CoA and acts as a base catalyst in enolization. The physiological reaction between acetyl-CoA and oxaloacetate can be clarified by applying the principle of 'catalysis by juxtaposition'. Cornforth, Redmond, Eggerer, Buckel & Gutschow (1969) and Lüthy, Rétey & Arigoni (1969) have investigated the stereochemistry of the aldol condensation involving acetyl-CoA and have demonstrated that the condensation occurs with inversion of configuration at the methyl group by using methyl groups made asymmetric with both deuterium and tritium atoms.

In contrast, the carboxylation reaction at the α -carbon of acyl-CoA takes place with retention of configuration, as elucidated in experiments on propionyl-CoA carboxylation (Rétey & Lynen, 1965).

The stereochemistry of ester condensation is not known. In studies on thiolase, the enzyme catalysing the reversible ester condensation of two molecules of acetyl-CoA:

it could be demonstrated that four thiol groups of the enzyme are involved in its catalytic activity and act as acyl acceptors in the overall process (Gehring, Riepertinger & Lynen, 1968; Gehring & Riepertinger, 1968). Tryptic digestion of labelled [$1\text{-}^{14}\text{C}$]acetyl-enzyme led to the isolation of a heptapeptide containing [$1\text{-}^{14}\text{C}$]acetylcysteine. The sequence of the peptide was found to be Val-Cys-Ala-Ser-Gly-Met-Lys (Gehring & Harris, 1968).

In fatty acid synthesis the condensation reaction leading to the formation of β -oxo acids occurs between sulphur-bound acetyl and malonyl residues (see Lynen, 1967):

No kinetic isotope effect was found in studies with deuterated malonyl-CoA as substrate (K. I. Arnstadt, unpublished work), indicating that CO₂ and the thiol (HSR) are eliminated in a concerted process.

The effects of thiol-blocking agents on the enzyme system involved in fatty acid synthesis will be discussed with particular emphasis to the participation of neighbouring groups of the enzyme in the condensation reaction.

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Selective Reactivity of Thiol Groups in Proteins

By K. WALLENFELS and B. EISELE. (*Chemisches Laboratorium der Universität Freiburg, Albertstrasse 21, Freiburg, Germany*)

The reactivity of the same functional groups in different proteins is known to vary to a great extent. The rates of alkylation of essential thiol groups in several enzymes range over five orders of magnitude (Eisele, 1967). In addition to the fact that groups can be masked by parts of the protein chains, these enormous differences may be due to activating or deactivating effects of neighbouring functional groups in suitable steric positions.

A simple model of a thiol group influenced by a neighbouring group is cysteine. The thermodynamic and kinetic parameters characterizing dissociation behaviour and nucleophilicity suggest a strong interaction between the thiol and amino groups of cysteine. Such a chemical system may be called a combined functional group (Wallenfels & Streffer, 1966).

An even higher order of combined functionality may be obtained with at least two groups differently influencing the reaction rate of a thiol group. In this case a centre of chirality with respect to the chemical reactions of the thiol group is formed. Protein thiol groups that are strongly influenced by neighbouring groups show high stereoselective preference for asymmetric reagents (Eisele, 1967; Wallenfels & Eisele, 1968; Eisele & Wallenfels, 1968a,b).

The thiol group of papain reacts with L(-)- α -iodopropionic acid 12 times as fast as with the D(+)-isomer. The reaction with α -iodopropionic acid is about 1000-fold faster with papain than with the thiol group of cysteine. The reaction rate with α -iodopropionamide with papain is only about 10 times that with cysteine. The stereochemistry of the reaction with the amide is opposite to that of the acid: D(+)- α -iodopropionamide reacts 4 times as fast as the L(-)-isomer. The alkylation data and their pH-dependence led to the hypothesis that a histidyl and a carboxyl residue influence the thiol group. As the absolute configuration of the asymmetric reagents is known, the chiral arrangement of the groups in the transition state of the alkylation reactions could be reconstructed (Wallenfels & Eisele, 1968). Independent X-ray work by Drenth, Jansonius, Koekoek, Swen & Wolters (1968) confirmed the proposed structure.

If the steric arrangement of groups in the neighbourhood of an active-centre thiol group is altered by a conformational change, the reactivity as well as the stereoselectivity of the thiol group will change. Conformational changes in the area of the active centre may be monitored by observation of changes in reactivity and/or stereoselectivity.

Glyceraldehyde phosphate dehydrogenase from yeast and from rabbit muscle are known to undergo conformational changes on addition of the ligand NAD⁺ (Kirschner, Eigen, Bittmann & Voigt, 1966; Conway & Koshland, 1968). The apoenzymes from both origins react faster with the D(+)-isomer of the free α -iodopropionic acid and the L(-)-isomer of the amide. On addition of NAD⁺ the stereoselectivity changes, now preferring the L(-)-isomer.

The results with the yeast enzyme could be interpreted in terms of the allosteric model of Monod, Wyman & Changeux (1965): the T state of the enzyme reacts most rapidly with the D(+)-isomers, whereas in the R state the L(-)-isomers of the reagents are preferred. The dependence of the reaction rates on the NAD⁺ concentration could be fitted to the allosteric function of state $\bar{R}$ (Eisele & Wallenfels, 1968b).

With the muscle enzyme the stereoselectivity for the L(-)-isomers is a discontinuous function of the NAD⁺ concentration: it is decreased by the first molecule of NAD⁺ bound to the tetrameric enzyme and then increases stepwise with the binding of three further NAD⁺ molecules. These results may easily be explained by sequential conformational changes induced by NAD⁺ binding as proposed by Conway & Koshland (1968).

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 Drenth, J., Jansonius, J. N., Koekoek, R., Swen, H. M. & Wolters, B. G. (1968). *Nature, Lond.*, **218**, 929.
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