PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 529th. Meeting of the Society was held at the University of Cambridge on Tuesday and Wednesday, 4 and 5 July 1972, when the following papers were presented:

COLLOQUIUM ON 'BEHAVIOUR OF REGULATORY ENZYMES OF CARBOHYDRATE METABOLISM'

Mechanisms for the Control of Enzymic Rates

By CARL FRIEDEN (*Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Mo. 63110, U.S.A.*)

Although the rate-limiting step in an enzymic reaction may be the chemical transition of substrate into product, there appear to be a number of other possible steps that may serve to control the rate of product formation. For example, the dissociation of the product from the enzyme-product complex may be the rate-limiting process of the overall reaction for several enzymes. Another step that may serve to control product formation is the isomerization of enzyme and/or substrate into a kinetically different form. If such isomerization occurs rapidly relative to product formation, one generates models for enzyme kinetic equations that reflect ligand-binding equations. If such isomerizations occur slowly or at rates similar to product formation (hysteresis), equations based on ligand-binding data will not adequately portray the kinetic data and the velocity of the reaction may not be a simple function of time. Since ligands themselves (either substrates or modifiers) may influence the rate of the enzyme isomerization processes, it might be expected that rate data for such enzymes would be rather complex. This may be particularly true in a series of metabolic steps where the concentrations of metabolites change continuously.

Rate-limiting intramolecular processes that are ligand-induced and that give rise to kinetically different forms of an enzyme may be detected by stopped-flow experiments in which product formation is followed for the whole time-course of the reaction. An example to be discussed is the bovine liver glutamate dehydrogenase where, if the time-course of NADH disappearance is followed, it can be shown that NADH binding to a second non-active site results in an inhibited form of the enzyme. The time-course of the conformation change induced by this binding is such that it is rapid relative to product formation at low enzyme concentrations but slow at high enzyme concentrations. Thus the kinetic properties of the enzyme depend on enzyme concentration. In other cases it might be expected that the conformational changes giving rise to kinetically different forms of the enzyme would be induced by allosteric

ligands, and the specific example illustrated by the glutamate dehydrogenase may be applicable to a large number of other regulatory systems.

Structure and Function of Liver Fructose 1,6-Diphosphatase

By S. PONTREMOLI (*Istituto di Chimica Biologica, Università di Genova, 16132 Genova, Italy*)

The specific fructose 1,6-diphosphatase from mammalian liver is known to be one of the enzymes required for gluconeogenesis.

Control of the phosphatase activity is provided by inhibition by AMP as well as by factors modulating the sensitivity of the enzyme to the AMP effect.

Several purification procedures of fructose 1,6-diphosphatase have been described.

The previously investigated homogeneous enzyme was described by us as 'alkaline' fructose 1,6-diphosphatase, as it shows a pH optimum near 9.0 and little activity at neutral pH.

The 'alkaline' enzyme has a molecular weight of 130000 and consists of four subunits of two different species (whose molecular weights are 29000 and 36000), as confirmed by analysis of the C-terminal amino acids.

The number of binding sites present in the enzyme molecule for the various ligands (AMP, fructose 1,6-diphosphate, Mn^{2+}) has been estimated to be four.

A possible control mechanism of fructose 1,6-diphosphatase is suggested by significant changes in the pH-activity profile as a result of a disulphide exchange demonstrated *in vitro* between the enzyme and biological compounds such as cystamine, CoA, acyl-carrier protein and homocystine.

The overall picture that emerges is that of a tetrameric protein with four binding sites for each ligand (one per subunit?) and with four highly reactive thiol groups possibly involved, when bound in disulphide linkage to certain biological compounds, in conformational changes required for maximum activity in the neutral pH range.

We have recently succeeded in isolating a new form of liver fructose 1,6-diphosphatase, with optimal activity at neutral pH, that was termed 'neutral or

native' fructose 1,6-diphosphatase (Traniello *et al.*, 1971).

The molecular weight of the 'neutral' enzyme has been estimated to be 144000. This enzyme form is made up of four identical subunits, as confirmed also by the finding of 4 mol of alanine residues/mol of enzyme at the C-terminal end.

Additional differences between the 'alkaline' and the 'neutral' fructose 1,6-diphosphatases are a higher sensitivity of the 'neutral' form to inhibition by AMP and the presence of 4 mol of tryptophan residues/mol of 'neutral' enzyme, this amino acid being absent from the 'alkaline' fructose 1,6-diphosphatase.

Digestion of the 'neutral' enzyme with subtilisin induces a progressive change in the pH-activity profile, which becomes very similar to that of the 'alkaline' fructose 1,6-diphosphatase (Traniello *et al.*, 1972).

These modifications in catalytic activity are accompanied by changes in molecular structure. The initial subunit of 36000 molecular weight is progressively replaced by a smaller species with molecular weight approx. 29000.

During digestion a progressive decrease in tryptophan content was observed that paralleled the formation of the lighter subunit and the decrease in the sensitivity to inhibition by AMP.

For references on the 'alkaline' fructose 1,6-diphosphatase see Pontremoli & Horecker (1971) and Traniello *et al.* (1971, 1972).

Pontremoli, S. & Horecker, B. L. (1971) *Enzymes*, 3rd. edn., 4, 612-646

Traniello, S., Pontremoli, S., Tashima, Y. & Horecker, B. L. (1971) *Arch. Biochem. Biophys.* **146**, 161-166

Traniello, S., Melloni, E., Pontremoli, S., Sia, C. L. & Horecker, B. L. (1972) *Arch. Biochem. Biophys.* in the press

The Structure and Regulation of the Lactose Synthetase System

By KEITH BREW, JOHN B. C. FINDLAY and BALWANT S. KHATRA (*Department of Biochemistry, University of Leeds, 9 Hyde Terrace, Leeds LS2 9LS, U.K.*)

Lactose synthetase consists of a galactosyltransferase (UDP-galactose-*N*-acetylglucosamine β -4-galactosyltransferase) and the regulatory protein α -lactalbumin (Brodbeck & Ebner, 1966; Brew *et al.*, 1967). In isolation the galactosyltransferase has a very low affinity for glucose and it catalyses the synthesis of lactose only at high glucose concentrations. The enzyme is present in tissues other than the mammary gland and functions in the biosynthesis of glycoproteins. In the presence of α -lactalbumin, which is synthesized specifically by the mammary gland, the affinity for glucose is greatly increased and lactose synthesis occurs at physiological concentrations of

glucose (Brew *et al.*, 1967; Hill *et al.*, 1968; Klee & Klee, 1970; Fitzgerald *et al.*, 1970). α -Lactalbumin has no separate enzymic activity, but is homologous in amino acid sequence and probably similar in conformation to lysozyme (Brew *et al.*, 1970; Browne *et al.*, 1969).

From a kinetic investigation of the reactions catalysed by pure human galactosyltransferase in the absence and in the presence of various α -lactalbumins we find, in contrast with a report by Morrison & Ebner (1971), that α -lactalbumin binds to an enzyme-Mn²⁺-UDP-galactose complex before the binding of monosaccharide. The so-formed intermediate has a higher affinity for glucose and other monosaccharides than in the absence of α -lactalbumin. The complex effects produced by α -lactalbumin with *N*-acetylglucosamine as galactose acceptor (Brew *et al.*, 1967) can be interpreted in terms of a similar mechanism with an alternative pathway in which α -lactalbumin binds to an enzyme-Mn²⁺-UDP-galactose-*N*-acetylglucosamine complex, causing dead-end inhibition (B. S. Khatra & K. Brew, unpublished work).

Recently the complete amino acid sequences of two further α -lactalbumins have been determined (Findlay & Brew, 1972; Brew, 1972). These sequences clearly confirm the homology with lysozyme and show a high proportion of interspecies amino acid substitutions. Three major regions of sequence are conserved in the three α -lactalbumin sequences now known: residues 34-42, 50-55 and 100-108. In the proposed conformation for α -lactalbumin the conserved regions are situated around the cleft region in the molecule, supporting the view that this area may be involved in the function of α -lactalbumin. In contrast, many residues that are freely variable in α -lactalbumin correspond to residues that are essential for the functioning of lysozyme.

This and other current evidence that is relevant to the relationship of the structure of α -lactalbumins to their unique biological function will be discussed.

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Brew, K. (1972) *Eur. J. Biochem.* **27**, in the press

Brew, K., Vanaman, T. C. & Hill, R. L. (1967) *Proc. Nat. Acad. Sci. U.S.A.* **59**, 491

Brew, K., Castellino, F. J., Vanaman, T. C. & Hill, R. L. (1970) *J. Biol. Chem.* **245**, 4570

Brodbeck, U. & Ebner, K. E. (1966) *J. Biol. Chem.* **241**, 762

Browne, W. J., North, A. C. T., Phillips, D. C., Brew, K., Vanaman, T. C. & Hill, R. L. (1969) *J. Mol. Biol.* **42**, 65

Findlay, J. B. C. & Brew, K. (1972) *Eur. J. Biochem.* **27**, 65

Fitzgerald, D. K., Brodbeck, U., Kiyosawa, I., Mawal, R., Colvin, B. & Ebner, K. E. (1970) *J. Biol. Chem.* **245**, 2103