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).

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The Structure and Regulation of the Lactose Synthetase System

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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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Negative Co-operativity in Glutamate Dehydrogenase: Coenzyme-Binding Studies

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The active oligomer of ox liver glutamate dehydrogenase consists of six apparently identical polypeptide chains of molecular weight $5.7 \times 10^4 \pm 0.3 \times 10^4$ (Cassman & Schachman, 1971; Smith *et al.*, 1970). Glutamate enhances the fluorescence of enzyme-bound NADH and NADPH and also has a pronounced hypochromic effect on the absorption spectrum; spectrophotometric titrations as the abortive complex indicated one binding site per subunit for both reduced coenzymes and an active-centre equivalent weight of 5.7×10^4 (Egan & Dalziel, 1971). Kinetic studies of the oxidative deamination of glutamate showed a complex pattern of coenzyme activation with both NAD^+ and NADP^+ , suggesting negative interactions between the six active centres in the oligomer (Dalziel & Engel, 1968; Engel & Dalziel, 1969). Equilibrium dialysis studies of NAD^+ and NADP^+ binding in the presence of the substrate analogue glutarate established that coenzyme binding in the ternary complex could not be described in terms of six identical and independent binding sites (Dalziel & Egan, 1972). Either there are negative interactions between the subunits or there are at least two sets of subunits with different intrinsic dissociation constants for coenzyme. In the presence of the allosteric effector ADP the coenzyme binding was weakened and could be described by a single dissociation constant.

Further information to elucidate the complex overall kinetics and the possible role of negative co-operativity has been sought by 'burst' experiments by the stopped-flow method and by reduced-coenzyme-binding studies by fluorescence titrations, utilizing both titrations of enzyme with coenzyme and titrations of coenzyme with enzyme. Taken together, both kinds of titration show conclusively that in the binding of NADH either there are negative interactions between the six subunits or about half the active centres bind coenzyme with a dissociation constant of about $2 \mu\text{M}$ and the remainder with a dissociation constant of $20 \mu\text{M}$. In the presence of glutamate qualitatively similar results were obtained, indicating one coenzyme-binding site per subunit, but

coenzyme binding was greatly strengthened and could be described by two dissociation constants of about 0.03 and $0.4 \mu\text{M}$. In presence of the allosteric effector GTP the coenzyme-binding curve was sigmoid, and the presence of more than one binding site per subunit was indicated. The latter result supports conclusions from circular-dichroism measurements (Jallon & Iwatsubo, 1971; Koberstein & Sund, 1971) and shows that the fluorescence of NADH bound at the additional site is also enhanced. Since GTP and NADH together cause dissociation of aggregates of the oligomer, a possible explanation of the apparent positive interactions in coenzyme binding is that the free oligomer has greater affinity for NADH than has the molecular aggregate.

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Some Properties of Yeast Isocitrate Dehydrogenase

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The activity of yeast isocitrate dehydrogenase is regulated by AMP, which increases the affinity of the enzyme for its substrates without altering the maximal velocity. The co-operativity of NAD^+ binding is diminished by raised concentrations of AMP or isocitrate. However, AMP activation causes only a slight fall in the Hill coefficient for isocitrate despite a 12-fold increase in isocitrate-binding affinity (Atkinson *et al.*, 1965).

The enzyme can be purified to homogeneity with 50% overall recovery by chromatography on DEAE-cellulose followed by selective elution from CM-cellulose by AMP. The isoelectric point of the enzyme rises from 5.6 to 7.0 during purification. This change can be revealed by isoelectric focusing, and is responsible for marked alterations in the behaviour of the enzyme on ion-exchange celluloses. Isoelectric focusing also generates a number of 'isoenzymes' as artifacts. These sub-forms cannot be discerned in any separation system that avoids the use of carrier