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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 $5.7\times10^4\pm0.3\times10^4$ (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 $2\mu M$ and the remainder with a dissociation constant of $20\mu M$. 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 $0.4\mu M$. 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