PROTEINS

Enzyme and Means

from our Molecular Biology Correspondent

FRIEDEN's group in particular has helped to establish bovine glutamate dehydrogenase as an archetype for a ligand-controlled enzyme. Moreover, a great number of physical methods have been brought to bear on the problem of identifying the various enzymatic manifestations with physical changes in the protein, in respect particularly of conformation and association state. The enzyme possesses both glutamate and alanine dehydrogenase activities. The first is activated by ADP, which is also known to promote the well known propensity of the molecule to associate indefinitely to a highly polymerized form. GTP, on the other hand, tends to dissociate the enzyme, while inhibiting glutamate dehydrogenase and stimulating alanine dehydrogenase activity. The evidence has not favoured the original conjecture that the glutamate dehydrogenase activity was associated solely with the polymer, but the issue has only now been conclusively settled.

R. Cohen has devised a method of observing the sedimentation of an enzymatically active species in the ultracentrifuge in minute concentration. It involves the use of a substrate, which on exposure to the enzyme causes an adsorption band to appear or to vanish, according to whether the substrate or the product is the accessibly absorbing species. A layer of enzyme solution is allowed to sediment in a band-forming centrepiece through the substrate solution. The ins and outs of the technique are described by Cohen and Mire (*Eur. J. Biochem.*, **23**, 267 ; 1971), and it clearly has great advantages, including the low requirement in material, the possibility of observing the enzyme in question in a protein mixture, and of operating at protein concentrations so low as to be otherwise non-existent from the viewpoint of the ultracentrifuge. Diffusion coefficients can also be determined. In the accompanying article (*ibid.*, 276) Cohen and Mire show results for a series of enzymes, including some not yet isolated in pure state, and determine the sedimentation coefficients of the active oligomeric states. There are no surprises, and for glutamate dehydrogenase in particular both glutamate and alanine dehydrogenase activities sediment at about 13S, which corresponds to the unassociated hexamer, which, according to hydrodynamic analyses of the association equilibrium, is the expected state at such low concentrations.

Arnold and Maier (*Biochim. Biophys. Acta*, **251**, 133 ; 1971) have isolated, crystallized and characterized glutamate dehydrogenase from rat liver. In terms of its sedimentation coefficient, and its reactivity towards antibodies directed to the bovine enzyme, it is indistinguishable from the latter. There is, however, no trace of the self-association behaviour. It seems by no means unlikely that this phenomenon is in fact a red herring so far as the physiological function of glutamate dehydrogenase is concerned.

Another issue which has been a matter of dispute for some time is whether glutamate dehydrogenase has one, or more than one, functionally important binding site per monomer for its coenzyme, NAD or NADH. The presence of two sites has now been inferred from circular dichroism measurements by Jallon and Iwatsubo (*Biochem. Biophys. Res. Commun.*, **45**, 964 ; 1971), closely followed by Koberstein and Sund (*FEBS Lett.*, **19**, 149 ; 1971). NADH, when it binds to the enzyme, greatly enhances optical activity, in the form of a sizable positive Cotton effect in its near-ultraviolet absorption band. In the presence of the modifying ligand, GTP, or of zinc ions, which exert the same kind of functional directing effect, this Cotton effect suffers an invasion and enhancement. A similar effect is brought about by the substrate, glutamate, in the presence of NADH. In the quaternary complex with substrate, NADH and zinc, however, the amplitude of the Cotton effect shows a further increase.

Observation of the ellipticity as a function of the NADH concentration in the presence or absence of substrate and activator indicates that the cofactor does indeed attach at two sites. The first and stronger is associated with a Cotton effect, that is modified in sense and magnitude by GTP or zinc ions, both of which may well interact directly with the NADH, its binding site, or both at once. The diphosphate, NADPH, will bind at only one site. ADP, when added to the ternary complex, chases the second NADH from its site. The suggestion of Jallon and Iwatsubo is that the second NADH site is made manifest by a conformational change resulting from the binding of the effector ligand.

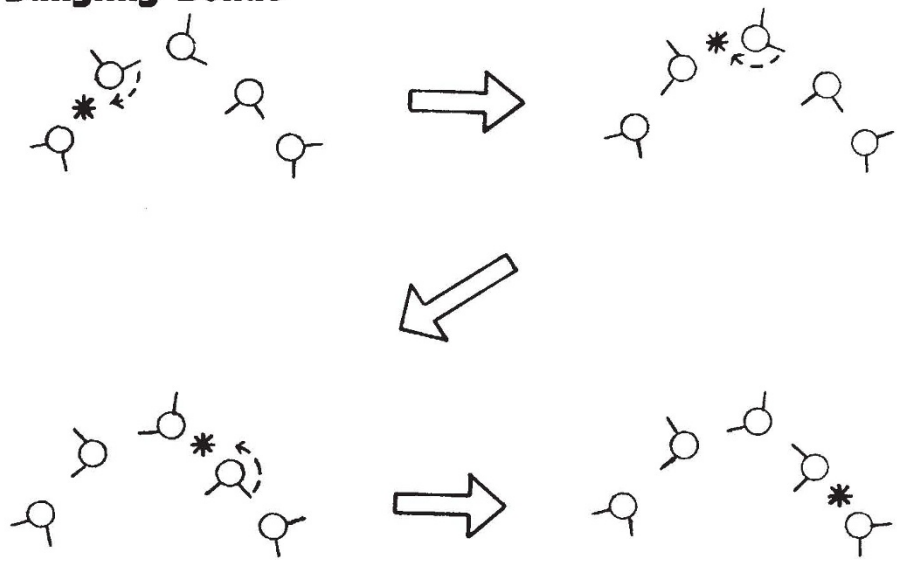
PHOTOBIOLOGY

Light and Life

from a Correspondent

THE use of damaging radiation on biologically active molecules was the central topic of a joint meeting on December 7 at the Royal Institution in London of the British Photobiology Society and the Society of Chemical Industry. It was clear from the remarks of Dr A. Knowles (University of Sussex) that low energy solar radiation not absorbed directly by a biological system can be very destructively utilized in a photodegradation reaction if an appropriate sensitizer is present. Under most conditions the triplet state

Dangling Bonds



IN next Monday's *Nature Physical Science*, Dr A. P. Hinton of the National Institutes of Health, Bethesda, presents computer calculations of the relaxation of the electric dipole moments of water in terms of a point defect model. The model is based on the fact that although the O-H--O bond is flexible there are some O-H groups that do not participate in a hydrogen bond.

These dangling bonds are considered by Hinton as being analogous to a crystalline point defect. The figure here shows a schematic representation of the migration of such a dangling bond by means of sequential reorientation of water molecules.