MOLECULAR BIOLOGY

Complicated Enzymes

from our Molecular Biology Correspondent

A FEW enzymes have been described recently which seem to possess two quite distinct catalytic activities. This phenomenon occurs in situations which demand parallel control of two synthetic processes by a single end-product, for example. Among the most interesting and best documented cases of such enzymes, by virtue of the work of G. N. Cohen and his group, is the homoserine dehydrogenase–aspartokinase, isolated from a strain of *E. coli*. The two activities are both subject to regulation by a single ligand species, namely L-threonine. The most recent report (Truffa-Bachi *et al.*, *Europ. J. Biochem.*, **7**, 40; 1969) is a study of the subunit structure of the protein. The molecular weight of the intact enzyme, from sedimentation equilibrium, is 360,000. Careful sedimentation equilibrium studies in 6 M guanidine hydrochloride give a subunit molecular weight of 60,000, and a similar value is obtained from the sedimentation coefficient in the same solvent, using the molecular weight-sedimentation coefficient calibration for this solvent derived by Tanford. End-group determination produced six N-terminal methionines, so that the structure has six polypeptide chains.

Truffa-Bachi *et al.* have gone further, and obtained fingerprint patterns of the protein: they are able to count fifty-two spots in the fingerprint; now it follows from the amino-acid analysis that there should be about 319 trypsin-labile bonds per molecule of 360,000, or—if the chains are identical—fifty-four per chain. Further, the protein contains twelve tryptophan residues, and staining with a tryptophan-specific reagent reveals just two tryptophan-containing fragments. Another good experiment involves tryptic digestion after acylation of the lysine residues, which renders the adjacent peptide bond resistant to trypsin. This leaves thirty-two labile bonds (at the arginines) per chain, if again the chains are identical, and twenty-eight spots are detected in the fingerprint. This work therefore shows as closely as is possible with these techniques that the chains really are identical. It may be noted that this is one of the relatively few proteins that contain both cysteine and cystine residues; the latter are shown to be intrachain. The numbers of binding sites on the intact enzyme have not yet been determined, but a structure involving two active sites and a regulator site on each subunit is likely. It should be said that the same workers have two mutant forms of the enzyme, both of molecular weight 180,000, one of which is devoid of homoserine dehydrogenase activity.

An enzyme which has received much attention as an archetypal allosteric system is muscle phosphorylase *b*. Its behaviour in relation to substrate and activator binding is complex (two sets of homotropic interactions being present), and it is in the best traditions of enzymology to combat the complexity by introducing as many independent parameters as may be needed to reconcile data and theory. The latest analysis, by Kastenschmidt *et al.* (*Biochemistry*, **7**, 4543; 1969), despite a good number of assumptions, however, carries considerable conviction. It is supposed from the outset that the transition between the conformational states of the enzyme is a fully "concerted" or all-or-none process. To account for independent co-operative binding of the activator, 5′-AMP, and the substrate, glucose-1-phosphate (though this latter depends on the buffer salts present), not one, but two high-affinity (R) states are postulated, as well as the low-affinity T state. The R' form binds AMP more strongly than does R, and glucose-1-phosphate less strongly. The T state does not bind AMP at all, and the substrate only weakly. The concentration of AMP then determines the equilibrium between R and R', and the formation of R' at low AMP levels formally explains the anomalously low activity observed in these conditions. The situation is further complicated by a dimer-tetramer equilibrium involving differences in binding constants, and the whole story with all its ramifications is highly involved.

Simultaneously with this work, Black and Wang (*J. Biol. Chem.*, **243**, 5892; 1968) have suggested from quite different evidence that phosphorylase *b* can exist in a third conformational state, which again has low affinity for glucose-1-phosphate. They have observed that IMP, which differs by only an amino group from AMP, is an activator; however, its primary effect is on the value of V_{max}, rather than on the substrate binding constant. It is suggested that there are two allosteric transitions from the T state, corresponding to a change in catalytic efficiency on the one hand, and binding strength on the other; IMP, unlike AMP, can effect only one of these changes.

MICROBIOLOGY

Rennin Substitute

from our Microbiology Correspondent

THE availability of the milk-clotting enzyme rennin is an important limitation of increased world cheese production. The enzyme is produced only in the stomachs of milk-fed calves, a source that clearly restricts increased yields. Consequently, a great deal of effort has been put into the search for an acceptable rennin substitute, and bacteria, fungi and even higher plants have been screened for such activity. These investigations had few results of practical value, although the rennin-like enzymes were found to be quite widely distributed—a finding that has encouraged further research. It is not surprising, therefore, to learn that at least two groups have now produced very promising microbial rennin substitutes, both products of phycomycetous fungi. After screening several hundred micro-organisms, Arima's group at the University of Tokyo finally isolated from soil a strain of *Mucor pusillus* that possessed a milk-clotting enzyme with the required properties. A preview of the *Mucor* enzyme was given at the International Fermentation Symposium held at Rutgers last September and now a fuller description has been printed (Yu, Tamura and Arima, *Biochim. Biophys. Acta*, **171**, 138; 1969). The Japanese team grew their mould on wheat bran and produced a good yield of crystalline enzyme preparation. The *Mucor* rennin has been used to produce various types of cheese using conventional cheese manufacturing steps and has passed these trials satisfactorily. It appears to have a molecular weight of about 30,000, a value similar to that of animal rennin, and other physical properties of the two enzymes are not greatly different.