NEWS AND VIEWS

Crystallographers' Progress

Now that the first shining successes of protein crystallography, lauded in song and story, have passed into history, the applause that greets the unveiling of each new model of a hydrolytic enzyme is becoming a trifle perfunctory. To stimulate the jaded intellectual palate something more overtly spectacular is evidently needed, and on p. 1098 of this issue of *Nature* we are happy to present just such a confection. Rossmann and his team have achieved a 2·8 Å map of no less an enzyme than lactate dehydrogenase, which has a molecular weight of 140,000, with more than three hundred residues in each of the four subunits. The reader, contemplating the stereograms with sagging jaw, might wish to be reminded that NAD is a cofactor for this enzyme, and that it binds independently to one site on each subunit. The high-resolution structure that Adams *et al.* now present is that of the apoenzyme.

The subunits have a compact form, with a cleft down the middle, containing the binding site for the adenylate function of the NAD, which, as recent work by McPherson from the same laboratory has shown, acts as a conformational trigger: its attachment engenders a structural transition to a state with high affinity for the nicotinamide end of the cofactor. The dodecapeptide that has been snipped out of the protein and contains the enzymically active thiol group has been identified, and lines one wall of the cleft. About a quarter of the residues are distributed between eight α-helical segments, and for connoisseurs of polypeptide structure there are some fragments of the rare and desirable 310-helix; 10–15 per cent of the residues are also in the β-form, some in the antiparallel, and some in the parallel form, which has never been found in isolation (as in fibrous proteins), but has been observed in the subtilisin molecule by Kraut and his coworkers. Most of the recognizable secondary structure occurs in the N-terminal half of the chain. Side-chains which are in a position to make contact with the cofactor have been tentatively identified, and Adams *et al.* note that some residues known to be functionally important in another dehydrogenase enzyme, glyceraldehyde 3′-phosphate dehydrogenase, occupy similar positions in the sequence to some that cluster round the cleft in the lactate dehydrogenase subunits. On this basis one might hazard the surmise that the stereochemistry of the active site could well be similar in the two species.

The real pay-off from this work, of course, lies in the future, when the holoenzyme and its inhibitor complexes are also solved at atomic resolution, so as to allow a detailed comparison. From the available data at 5 Å, the general form of the conformational convulsion that accompanies the binding of the cofactor has already been defined (Adams *et al.*, *J. Mol. Biol.*, **51**, 31; 1970). In their new report in this issue, the authors also describe the important step of crystallizing an abortive complex of the holoenzyme with the substrate, pyruvate. From the results at 5 Å, it is already clear that the introduction of the latter brings about a considerable displacement in one part of the molecule —a loop of twenty-four residues making up the mouth of the cleft. This migrates 12 Å, thereby closing the cleft so as to occlude the nicotinamide ring. A change in electron density also occurs in the vicinity of asn-150 and ser-147, which form part of the fragment containing the essential thiol group. Enzymologists will wish Rossman and his colleagues a swift ascent to the next daunting peak.

PROTEINS

Independence and Togetherness

from our Molecular Biology Correspondent

THE problem of cooperative binding phenomena in proteins, which not so long ago seemed on the verge of illumination in terms of a number of simple generalities, has now, alas, receded once more into a malodorous miasma of complex and contradictory observations. Even the archetype, haemoglobin, which has probably claimed more man-years of research than any other protein, has not been spared. A fresh attempt to impose a simple formalism on the kinetics and equilibrium of the oxygenation reaction has now been made by Gibson (*J. Biol. Chem.*, **245**, 3285; 1970). He has expressed the uptake of oxygen by the four haems in terms of four independent consecutive steps. The data, in other words, must be accommodated by eight rate-constants, four each for the forward and reverse reactions. Now in terms of the varyingly attributed quip that with four independent parameters one can draw an elephant, this seems at first sight a dispensable exercise. But, on closer inspection, a number of restraints reveal themselves. In the first place, the rate constants for the reaction of the fourth oxygen can be independently determined from measurements of the kinetics of replacement of oxygen by carbon monoxide, the carbon monoxide system being kinetically defined by the early work of Roughton and Gibson. The remaining six rate constants must then be selected so as to satisfy a family of curves for the binding and dissociation of oxygen at different levels of saturation. The ratios of the on and off-rate constants must, moreover, be such as to yield binding constants which will fit the equilibrium curve.

These requirements between them are sufficiently restrictive that a set of what one may hope are unique rate constants can be computed. Gibson concedes that he has defined the system only in operational terms, and that more states than the five which are implicit in this scheme must be presumed to exist. It is, however, seen that the cooperative binding of oxygen can be accounted for by the different values of the rate constants. Moreover, their relative magni-