02. STRUCTURAL MOLECULAR BIOLOGY

02.X-11 PROTEIN ENGINEERING: TECHNICAL AND COMMERCIAL PROSPECTS. By Brian S. Hartley, Centre for Biotechnology, Imperial College, London SW7 2AZ.

To protein chemists, the prospects offered by site-directed mutagenesis of cloned genes and high-level expression in microbial cultures are mind-boggling. We have learned to try to make good guesses relating specific structures to observable properties of whole classes of enzymes, but the experimentalist taches to change specific residues and predict altered properties. This is now possible and must open a new chapter in enzymology.

However we are all under increasing pressure to be commercially relevant, and the biotechnologist has to ask more pragmatic questions: What can be done when? What will it cost? What are the chances of success? What will the product earn? There is some danger that overall the commercial prospects may prejudice the ordered accumulation of basic data necessary for steady development of the art. Nevertheless some prospects are reasonably short-term, e.g., modifications of major industrial enzymes to alter stability, catalytic activity, specificity or pH dependence. De novo design of new enzyme specificities, modification of natural enzymes to alter stability, catalytic activity but the pH optimum has apparently shifted to pH 3.5 verses pH 7.0 for the wild type. Although we anticipated that the pro39 to cys mutant would contain all the cysteines as sulfhydryls. Several protocols for oxidation have failed to produce a homogeneous disulfide. The enzyme is not grossly unfolded since it has no detectable enzymatic activity but the pH optimum has apparently shifted to pH 7.5 verses pH 7.0 for the wild type. Although we anticipated that the pro39 to cys mutant would contain all the cysteines as sulfhydryls. Several protocols for oxidation have failed to produce a homogeneous disulfide. The enzyme is not grossly unfolded since it still contains the active site thiol.

02.X-12 DIRECTED MUTAGENESIS OF DIHYDROFOLATE REDUCTASE. Jesus E. Villafranca, Elizabeth E. Howell, John M. Abelson, and Joseph Kraut. The Agouron Institute, La Jolla, California.

Three mutations of the enzyme dihydrofolate reductase (DHFR) were constructed by oligonucleotide-directed mutagenesis of the cloned E. coli gene (Villafranca, et al. Science 222, 782-788 (1983)). The mutations - at residue 27, aspartate replaced with asparagine; at residue 59, proline replaced with cysteine; and at residue 95, glycine replaced with alanine - were engineered to answer questions raised by the x-ray structure about the enzymic mechanism and certain aspects of chain folding and dynamics. The mutant DHFRs have been purified and partially characterized. In the case of the asn27 mutant, the enzyme, as expected, does not protonate methotrexate in the binary complex although the binding constant is lowered by only 100 fold. Surprisingly, the asn27 enzyme retains 1/1000th of the enzymatic activity but the pH optimum has apparently shifted to pH 3.5 verses pH 7.0 for the wild type. Although we anticipated that the pro39 to cys mutant would contain all the cysteines as sulfhydryls. Several protocols for oxidation have failed to produce a homogeneous disulfide. The enzyme is not grossly unfolded since it still contains the active site thiol.


The structure and dynamics of water occurring in its disordered state around biomolecules and in bulk liquid are not well understood at the molecular level. To investigate these problems, the distribution of the water molecules in crystals of the vitamin B12 coenzyme (60-70 waters per unit cell) has been analysed, using both high resolution neutron and X-ray diffraction. Three sets of data were measured: neutron to 0.95A resolution, and 2 sets of X-ray data to 1.07 and 0.92A resolution. The corresponding models were extensively refined using the methods of least squares and Fourier synthesis. The final R-factors were 0.085, 0.086 and 0.134 respectively. The solvent regions in these 3 models were examined and during the refinement many water sites were identified - most of which appeared to be disordered. An acetone molecule was also located. The individual solvent sites within each of the 3 models, along with those assigned in the 1968 X-ray model (P.G. Lauter, Proc.Roy.Soc. A303, (1968)) were compared and many were seen to be located in similar positions in the 3 different models. The analysis of the solvent regions proceeded in two stages: Firstly, several different water networks were formulated from the better defined stable sites and these were seen to extend throughout all the solvent regions of the crystal. Secondly, some of the dynamical aspects of the solvent structure were examined from the time averaged experiments, in terms of the continuous solvent density observed around and between the stable sites.

The interactions of the water molecules (stable and disordered) with both the coenzyme groups (polar and non-polar) and the other water molecules have been studied with respect to their mutual geometrical arrangements. Indications are that, in addition to participating in standard 2 centred hydrogen bonds, they are also involved in 3 centered interactions, where the non-covalent H...X donor-acceptor distances lie within the range of 2.0 to 2.6A. At these longer distances (end thus weaker interactions), the waters are seen to be more disordered and movements of the individual molecules over small distances can be observed in the solvent density. They appear to move in a cooperative manner with some of them 'breaking away' from the more strongly bound sites. Monte Carlo computer simulation calculations have also been performed for the solvent in this macromolecular system and the results are described by F. Vovelle, J. L. Finney, and P. Barnes in this volume.