Design, construction and properties of novel protein molecules

This discussion meeting was held under the auspices of the Royal Society at Carlton House Terrace on the 5 and 6 June 1985. The meeting was a huge success and drew the largest attendance ever (500) to an RS meeting of this kind. Only 300 people could be seated in the main lecture theatre, so there was a video relay for the extra 200.

M Smith opened the meeting by describing experimental mutagenesis techniques, including the introduction of random point mutations by chemical methods, specific mutagenesis with the use of oligodeoxyribonucleotides to mutate wild-type genes via heteroduplex structures, and partial or total gene synthesis from oligonucleotides to construct both strands of a DNA molecule.

Mutagenesis of tyrosyl tRNA synthetase as a probe of hydrogen bonding in proteins was described by Alan Fersht. Mutagenesis of the enzyme to delete a hydrogen bond between the enzyme and substrate was shown to weaken binding by around 4 $kJmol^{-1}$ if the now unpaired donor/acceptor is uncharged and approximately 17 $kJmol^{-1}$ if the unpaired ensemble is charged.

Brian Hartley gave an overview of the commercially important enzymes and the economics of their production. Cost and operating stability are key elements for industrial enzymes. Genetic engineering offers the possibility that the properties of industrially important enzymes can be tailored to fit process needs.

Jeremy Knowles described the use of directed mutagenesis as an aid to understanding the mechanism of action and free energy changes involved in catalysis by triosephosphate isomerase. The changes in the free energy profile resulting from changes in residues believed key to the catalytic function, Glu 165, His 95, Lys 13, were discussed.

The subject of M Courtney's talk concerned the inhibition of serine proteases by α_1-antitrypsin (α_1-AT). Directed mutagenesis of a cloned α_1-AT gene permitted analysis of the relation between the inhibitor reactive centre and its ability to inactivate various proteases. Possible therapeutic uses of such mutant inhibitors in the treatment of emphysema and as anticoagulants were discussed.

R N Perham investigated the pyruvate dehydrogenase multienzyme complex of *E. coli*. The effect of selective deletions in the dihydrolipoamide acetyltransferase gene to create truncated chains, which were then modified by site-directed mutagenesis, on the assembly, catalytic activity and active site coupling in the complex were discussed.

J E Villafranca's laboratory is working on mutant dihydrofolate reductases, where mutations at the active site have revealed that Asp 27 is involved in substrate protonation and not transition-state stabilization as previously thought. Substitution of Pro 39 by a Cys places two sulphydryls in a position to form a disulphide bond under oxidizing conditions. The resulting mutant DHFR is significantly more stable against guanidine HCl or urea denaturants than the wild-type enzyme.

After the genetic engineering talks, the theme of the discussion moved on to molecular graphics in the structure determination of proteins and the 3D design of mutant proteins. Tom Blundell gave an overview of the various techniques involved, which were then expanded upon by the other speakers.

Arthur Lesk and Cyrus Chothia discussed the conformational changes induced in proteins by amino-acid mutations, insertions, or deletions. Loops on the surface can often accommodate these changes by local refolding. Mutations involving the buried hydrophobic residues generally result in rigid body movement of helices and sheets. The nature of the forces that stabilize protein structures sets limits on the conformational changes.

Bob Langridge illustrated the use of a variety of computational techniques to solve problems in macromolecular chemistry. The structural changes related to directed mutagenesis are best modelled with computer graphics and molecular mechanics calculations on a number-crunching computer. On the other hand, research on protein folding involved the use of declarative languages and symbolic computing.

David White described the building of a model of human thrombin from the X-ray crystal structure of α-chymotrypsin using a molecular modelling system. Amino-acid substitutions, deletions and insertions were modelled automatically by means of a list of rules relating to protein structure and a hierarchical energy minimization scheme. The use of models so constructed for enzyme/substrate docking studies in drug design was discussed.

The final session of the meeting returned to the topic of specific applications of directed mutagenesis with a talk by J A Wells on the alteration of substrate specificity and stability in subtilisin. The gene for subtilisin was cloned and amino-acid substitutions made at positions 222, in an attempt to enhance stability, and 166, in an attempt to enhance specificity.

M S Neuberger introduced us to monoclonal antibodies and described the production of chimaeric antibodies, with mouse-encoded

antigen-binding variable regions coupled to constant regions with human physiological effector functions, by exon shuffling. Site specific mutagenesis allowed a detailed analysis of the structural basis of the interactions of antibodies with their antigens or their effector proteins.

The last scheduled talk was by Greg Winter on the protein engineering of tyrosyl tRNA synthetase. Over 100 mutants of the enzyme have been made and these have allowed the evaluation of the precise roles of side chains in substrate binding. The mutations of lysine and arginine residues have allowed the mapping of the tRNA across the surface of the synthetase while other mutations have resulted in improved catalytic rate, substrate affinity, enzyme specificity and the cleavage of the dimeric enzyme into two monomers.

There then followed two short unscheduled talks from members of David Blow's and Max Perutz's laboratories and the summing up by Max Perutz in which he concluded that this was the best scientific meeting that he had been to. I wholeheartedly agree with this assessment and David Blow, Alan Fersht and Greg Winter are to be congratulated for arranging this masterpiece.

David White

BOOK REVIEW

A pictorial approach to molecular structure and reactivity

R F Hout Jr., W J Pietro and W J Hehre, John Wiley, UK (1984) £40.85, 403pp

With the advent of more powerful computers, the area of quantum chemistry has enjoyed a considerable interest and expansion over the past couple of decades. There is a large amount of information contained in the wavefunction that is calculated by quantum chemistry programs, and one of the problems is how best to represent it. The most basic and perhaps least attractive method, since it involves quite a bit of work, is simply to draw out, with pen and paper, diagrams of the molecular orbitals. This is where this book steps in and essentially removes the hard grind, by presenting the results for a number of simple inorganic and organic systems by means of a set of monochrome photographs of the molecular orbitals taken from a VDU screen. At first glance this would appear to be a godsend; however, the picture quality leaves a lot to be desired.

The vast proportion of the book discusses the valence molecular orbital photographs of a number of systems. The text, what there is of it, is clear and to the point, although it is far removed from the relevant pictures, and so the reader has constantly to keep turning back the pages in order to find what is of interest in the pictures he is looking at. The first three chapters contain a brief overview of quantum mechanics and how the pictures are produced; the rest is taken up with the results.

Nowadays, computer graphics is a popular and attractive method for displaying molecular properties, be it a ball and stick model, a CPK type representation or electrostatic potential results. In all of these, colour is invariably used; however, in this volume only black and white photographs have been considered. There are problems in obtaining photographs from a VDU screen, and, unfortunately, in this case they have not been overcome. The pictures are dark, fuzzy and very difficult to interpret. It is hard to understand why they were produced on a black background, and certainly a white background would aid their interpretation considerably. It would have been even better to have used colour, but that presumably would price an already exorbitant book out of the market.

It is hard to say what sector the book is aimed at, since quantum chemists probably have a good idea of what the orbitals look like anyway and there are various other graphics packages that do a better job. Some of the pictures may be useful in undergraduate teaching, and so libraries might buy a copy as a reference work.

Overall, this is a very disappointing and not particularly useful book, although it is based on a good idea. It is unfortunate that the presentation (not the binding and paper quality, which is excellent, but rather the photographs) has let it down. Much more information, which is presented in a better form, can be obtained in Albright, Burdett and Whangbo's *Orbital interactions in chemistry*. The best place for this reviewed work is in some dusty corner of a bookcase where it can be forgotten for all time.

A F Cuthbertson