ENZYMES

Numinous Nuclease

from our Molecular Biology Correspondent

A **SIZABLE** textbook could now be written about bovine pancreatic ribonuclease: it would encapsulate the most striking achievements of modern enzymology, protein chemistry, and X-ray crystallography. Last year the total synthesis of active ribonuclease was reported simultaneously by two laboratories. The full details have now been described by Gutte and Merrifield (*J. Biol. Chem.*, **246**, 1922; 1971), in an article the length of which reflects the magnitude of the undertaking. It is mandatory reading for anybody interested in the state of the craft of solid-phase peptide synthesis — the procedure developed by Merrifield, and representing one of the most important technical advances in biochemistry of recent years.

The scope of the method is limited by the efficiency of each peptide addition step, and to a lesser extent the ease of cleavage of the finished product from the column support, and of the protecting groups from side chains. By dint of a variety of minor modifications, the loss at each addition cycle was brought down to a level of 1.4 per cent, which corresponded to a yield of the final product of 124 residues of 17 per cent. Cleavage from the resin and deprotection were accomplished in a single step with a maximal efficiency of 63 per cent. Oxidative formation of the correct disulphide pairings, followed by ion-exchange chromatography, gave a highly active product; this was then further fractionated by treatment with trypsin, to which native ribonuclease is highly resistant, so as to remove any incorrect, and by implication improperly folded, contaminants, and this was followed by a fractional ammonium sulphate precipitation. The resulting synthetic enzyme had an activity of 78 per cent, compared with the native crystalline preparations, and by a series of chemical and enzymological criteria the two were indistinguishable.

Some interesting applications of such syntheses are not slow to suggest themselves, and one is to prepare selectively aberrant chains, in which residues are changed, omitted or added at will. One such exercise is described by Gutte and Merrifield. Ribonuclease S is a form containing a break in the polypeptide chain between residues 20 and 21. Since the separated S-peptide (1-20) can be restored to its position in the protein with regain of activity, it has been possible to evaluate the contribution of particular side chains in the peptide towards correct folding and activity, by studying the interaction of S-protein with modified S-peptides. Gutte and

Merrifield have now instead modified the S-protein by terminating the synthesis after ninety-nine residues, thereby eliminating five residues, including one buried tyrosine. S-peptide not only restores the activity by combining with this truncated S-protein chain, but its presence also permits it to refold correctly from the reduced state. The missing pentapeptide has at least two residues that seem to be involved in a system of hydrogen bonds to other parts of the chain. Evidently a partial disorganization in this region of the enzyme has no effect on the integrity of the active site.

A method that has been widely used to observe interactions at the active site is proton magnetic resonance. A difficulty associated with this technique in general is to disentangle and assign resonances arising from particular side chains. A new approach, which serves to simplify the picture, and to visualize events only as they affect a particular side chain, has been attempted by Huestis and Raftery (*Biochemistry*, **10**, 1181; 1971). They have studied magnetic resonance of the fluorine nucleus, this being introduced into the protein by trifluoroacetylation of lysines. Both the lysines of S-peptide can be modified in this way, without detriment to its ability to recombine with S-protein. Three resonances, two from the ϵ -amino substituents and one from the α -amino, can be assigned, and the first two shift and one splits on recombination of the peptide with S-protein. Introduction of inhibitors, including the phosphate ion, causes the perturbation only of a signal from lys-7. From the structure of the enzyme, and the pH profile of the chemical shift, Huestis and Raftery deduce that the inhibitors effect a small displacement in the his-12 imidazole, which probably depends on the protonation state of the opposed active site

imidazole of his-119. This type of approach can also be used (perhaps as rather a hard way) to study binding equilibria. Lee and Chan (*Biochem. Biophys. Res. Commun.*, **43**, 142; 1971) have used ^{31}P nuclear magnetic resonance to observe the binding of UMP to ribonuclease. The broadening of the resonance from the inhibitor is used to obtain a binding profile and an association constant. The broadening is related to the rate of exchange of ligand at the binding site, and can be used to obtain a dissociation rate constant, which agrees moderately well with directly measured values.

INTERSTELLAR MOLECULES

Sulphur—at Last

ONE of the more elusive atoms in interstellar space has now been tracked down. In a recent *International Astronomical Union Circular* (No. 2322), P. M. Solomon, of Columbia University, reports observations of three new interstellar molecules, carried out in collaboration with K. Jefferts, A. Penzias and R. Wilson, of the Bell Telephone Laboratories.

Using the National Radio Astronomy Observatory's 36 foot (11 m) antenna at Kitt Peak, this team has found methyl cyanide (CH_3CN), carbonyl sulphide (OCS) and carbon monosulphide (CS) in some of the well studied interstellar clouds. The Sagittarius complex has again proved a fruitful source, with transitions corresponding to $J=6-5$ ($K=0$ and 1) for CH_3CN appearing in the spectra of both Sgr A and Sgr B2 at 110.383 GHz and 110.381 GHz.

But it is the long awaited discovery of sulphur which is the most interesting feature of this report. In the spectrum of Sgr B2 (but not in Sgr A) there is a feature attributed to the OCS

Thermal History of Lunar Rocks

AFTER examining a sample of lunar basalt, S. S. Hafner and his colleagues at the University of Chicago report in next Monday's *Nature Physical Science* that annealing of rock from the surface of the Moon took place at about 600° C. This is shown by sub-microscopic unmixing and ordering of Mg and Fe among the cation sites in the mineral which was present as large, probably equilibrium, crystals suspended in lava when it was erupted from the lunar surface at about 1,150° C. This effect is not observed in terrestrial lavas with simple cooling histories. Slow cooling in thick flows might lead to the observed effects, but more often leads to microscopic unmixing and physical separation of the two components from high temperature crystalline phases in

the original homogenous state. Similar effects are known in ancient terrestrial igneous rock, particularly in dykes cutting gneisses in continental shield regions, where the rock has been rapidly cooled and then reheated or held for long times at moderate temperatures.

At a recent International Astronomical Union symposium at New-castle upon Tyne (see *Nature*, **230**, 491; 1971), there was discussion of evidence showing the real age of lunar lavas to be 4.5×10^9 years, implying that the $3.3-3.7 \times 10^9$ year ages, widely reported for the formation of the groundmass minerals in the rocks, must refer to a subsequent thermal event for which Hafner *et al.*'s results might provide independent evidence.