PROTEINS

To Catch an Enzyme

from our Molecular Biology Correspondent

AFFINITY methods of dipping into a mixture of components and extracting only the one with the right activity are, conceptually, at least as old as immunology, but their full power and versatility are only now being properly exploited. The principle is obvious enough: a ligand, for example, is immobilized and used to trap the protein for which it is specific, or vice versa. Affinity chromatography has, for example, been central to the work of Anfinsen and his colleagues on the chemical synthesis of staphylococcal nuclease. A terminal peptide fragment can be split from the chain, which will reassociate non-covalently with the residuum of the molecule to give a fully active enzyme. Chemical synthesis of the sequence corresponding to the terminal fragment gives an impure product because of side reactions, but the functional article is recovered from the mixture by passage through a column to which the residual protein is covalently attached.

The same principle has now been adapted to the capture of affinity-labelled fragments of the same enzyme. In affinity labelling of active sites, a bifunctional molecule is added to the protein, one end being a specific ligand (for example, inhibitor) that attaches at the active centre, the other a group of high chemical reactivity, which will react with any suitable side chain within reach. Digestion of the protein labelled in this manner then produces fragments, one of which contains the label and at least part of the active site or its environs. Givol *et al.* (*Biochem. Biophys. Res. Commun.*, **38**, 825; 1970) have hit on a way of isolating the fragment which circumvents the tedium of peptide separation procedures. The point is merely that the fragment still contains the specific ligand group, and may therefore be trapped on a column of the selfsame enzyme. As an example of this quite general strategy they give the following: pancreatic ribonuclease can be affinity-labelled with a derivative of uridine monophosphate with a phenyldiazonium-containing group substituted in the sugar. This reacts specifically with a tyrosine residue by the mononucleotide-binding site. The enzyme thus labelled is reduced, alkylated and digested with trypsin. The mixture of peptides is passed through a column of agarose beads to which ribonuclease is covalently bound through its amino groups by a now standard procedure that has been used to produce matrix-bound, insoluble enzymes. The mononucleotide end of the labelled peptide binds to the enzyme, all other peptides are washed through and the trapped ligand is flushed out with dilute alkali, pure and concentrated. A similar procedure has been used to prepare a peptide fragment from affinity-labelled anti-DNP antibody.

The application of the method to the study of active site peptides of staphylococcal nuclease is by Wilchek (*FEBS Letters*, **7**, 161; 1970). The specific inhibitor is deoxythymidine diphosphate, and the bifunctional version of this is substituted at the phosphate by a bromoacetyl-*p*-aminophenyl group. The bromoacetyl function is very reactive and attaches to lysines. After reaction and fragmentation by trypsin, the peptides are passed through a column of the nuclease bound to agarose, and only the labelled fragment sticks. A

phenyldiazonium derivative has also been used in the same way, and reacts with a tyrosine residue. The peptide fragment was identified in terms of the position of its sequence in the chain, and it bears the label at Tyr-115, whereas the other reagent attaches to Lys-48 and Lys-49.

A more conventional application of affinity chromatography has been used by Gribnau *et al.* (*Biochem. Biophys. Res. Commun.*, **38**, 1064; 1970) for isolation of a protein inhibitor of ribonucleases. This comes from rat liver, and its partial purification by conventional methods has been a laborious matter. Pancreatic ribonuclease, anchored to a carboxymethylcellulose matrix (now a commercial product), was used to catch the specific inhibitor in the liver homogenate. After washing through the extraneous proteins, the enzyme-inhibitor complex was dissociated by high salt concentration. The product showed high activity, and was homogeneous by gel electrophoresis. Ribonucleases are the scourge of biochemists who operate in the general area of protein biosynthesis, and the rat liver inhibitor may prove to be of no small practical value.

POLYAMINES

Mysterious Functions

from a Correspondent

FORTY years after the election of S. O. Rosenheim to the Royal Society for discovering spermidine, and clarifying both its structure and that of spermine, the functions of these compounds and of their precursor, putrescine, are beginning to be understood. Recent advances in their physiology and biochemistry were discussed at a conference held on April 9 and 10 by the New York Academy of Sciences.

Drs A. B. Pardee and M. Inouye (Princeton University) described how putrescine and spermidine act in the synchronization of the division of amino-acid-deficient bacterial mutants. Dr W. K. Maas (New York University) described how division of putrescine-requiring strains of *E. coli* was selectively arrested in the absence of exogenous putrescine and spermidine. The genes for several steps in putrescine biosynthesis are clustered, suggesting that there is an operon for this function. Dr D. R. Morris (University of Washington) described the network of constitutive and inducible enzymes of putrescine biosynthesis in *E. coli*. The production of putrescine is subject to coarse controls of repression and derepression, and to fine controls of feedback inhibition and substrate availability. Dr S. S. Cohen (University of Pennsylvania) described the production of abnormally large amounts of spermidine in relaxed bacteria and of putrescine in organisms deficient in potassium ions. The latter phenomenon, which also occurs in plants, although involving different mechanisms of enzyme imbalance, was suggested by Dr T. A. Smith (Long Ashton Research Station, Bristol) to indicate the existence of a homeostatic mechanism operative in acidic soils or those deficient in potassium ions.

Dr L. Stevens (King's College, London) showed spermidine and spermine to be bound to cellular ribosomes. These polyamines were found by Cohen in tRNA isolated at low ionic strength. Detection in tRNA involved a new sensitive fluorometric method