in consequence of which some (the longest) chains change their conformation within a narrow range, while others have a broad transition profile. Observations of a specific interaction of the solvent component, trifluoroacetic acid (TFA) with the polypeptide are described by Liu and Lignowski (*ibid.*, 739), who find that the TFA protons themselves undergo large shifts in the transition region. This effect makes it possible to follow the transition even when the polymer is so large that its own line-widths are too broad to see.

The denaturation of proteins can also, of course, be observed by NMR, and Cohen-Addad (*J. Mol. Biol.*, **50**, 595; 1970) has devised a new criterion—the position of the centre of gravity of the cluster of resonances from the aromatic residues. With thermal denaturation the lines sharpen and shift away from their perturbed positions in the native protein. This shift then provides yet another criterion of the denaturation process, which reflects the average state of the aromatic residues. Boublik *et al.* (*Europ. J. Biochem.*, **14**, 486; 1970) have looked at the conformational states of different parts of the chain in aqueous solutions of two histone fractions of known sequence. Addition of salt leads to increased helicity, and this is accompanied by broadening of certain lines in the spectrum, which can be assigned to parts of the chain. By this means the conformational disturbance can be localized, though it is difficult to discriminate between the effects of helicity and aggregation at specific sites.

Perhaps the most successful applications of NMR have been in enzymology, and the work of Markley and Jardetzky (*J. Mol. Biol.*, **50**, 223; 1970) on the interaction of the nucleoside diphosphate inhibitor with staphylococcal nuclease is a good example. The histidine residues can be observed individually in the spectrum, and one of them has a double peak. It is inferred to exist in two environments, which represent conformationally distinct states of the enzyme. The equilibrium between them is displaced when inhibitor is added, presumably in favour of the form for which it has the higher affinity. By the use of an enzyme preparation containing selected deuterated amino-acids to simplify the spectrum, the tyrosine resonances could also be resolved, and three of them were seen to shift when the inhibitor was bound. These residues are thought to be involved directly in the binding, and in the complex there is evidence of a further conformational equilibrium manifested by a tyrosine. A complete description of the system has not yet been achieved, however.

In another enzyme, chymotrypsin, the binding of the aromatic inhibitor, *trans*-cinnamate, has been studied (Gerig and Reinheimer, *J. Amer. Chem. Soc.*, **92**, 3146; 1970) and is accompanied by marked broadening and shifts in the inhibitor spectrum, which are interpreted in terms of immobilization of the ring in close contact with an aromatic group in the protein. The lines from the vinyl end of the inhibitor by contrast are scarcely perturbed.

Shifts of striking magnitude are found in the protons of the haem group in ferric haem proteins, and arise from interactions with the iron, according to a well known mechanism. A study of a series of myoglobins, native and modified, by Wüthrich *et al.* (*J. Biol. Chem.*, **245**, 1947; 1970) leads to the reassuring conclusion that only amino-acid residues close to the haem group interact with it. Nevertheless, the spectra, at 220 MHz, are very pretty.

ENZYMES

Ribosomes make Esters

from our Biological Chemistry Correspondent

It is no chicken and egg problem to say that ribosomal peptidyl transferase is the enzyme which makes enzymes. Its job is the synthesis of all proteins translated from ribonucleic acid. Essentially it repeatedly promotes the reaction between a peptidyl ester and an amine to form a peptidyl-amide, itself a peptidyl ester which contains one more amino-acid.

Studies on the mechanism of action of peptidolytic enzymes, which have the reverse role, have made much use of their ability to hydrolyse esters as well as amides. This ability to use oxygen analogues has been demonstrated recently for the peptide-synthesis enzyme by a group at the Massachusetts Institute of Technology.

The antibiotic puromycin (I) is a well-known inhibitor of protein synthesis. It has a primary amine function (*R*) which closely resembles that of an amino-acyl-tRNA and readily attacks the ester link of a peptidyl-tRNA to halt ribosomal protein synthesis. Rich and his colleagues (*Biochemistry*, **9**, 2477; 1970) have made two active analogues of puromycin in which a hydroxyl group replaces this active amine. The first, hydroxypuromycin (II), is one of the products of nitrous acid deamination of (I). The second, ψ -hydroxypuromycin (III), is a chemically synthesized analogue.

Both of these alcohols react specifically with formyl-methionyl-tRNA in the presence of ribosomes to give the carboxylic esters of formylmethionine and the analogue. Although these reactions are ten times slower than that involving puromycin itself, several criteria are satisfied which support specific catalysis by the ribosomal enzyme. Preliminary results suggest that the enzyme uses a general-base function of pK_a between 7 and 8.

One of the fruits of this study is a speculation about the mechanism of termination in protein synthesis—which requires transfer of the completed peptide from the last tRNA to a water molecule. If the peptidyl transferase has sufficient flexibility to catalyse ester formation as an alternative to normal amide synthesis, Rich's results give credence to the hypothesis that an allosteric change in the enzyme could effect chain termination by making peptidyl transfer to water the dominant reaction catalysed by the ribosome.