POLYPEPTIDES

Polyproline Observed

from our Molecular Biology Correspondent

THE study of synthetic polypeptides is a gentlemanly pursuit which has been happily spared by the surging tide of molecular biology. At a remove, it nevertheless continues to provide lessons for the protein chemist, and the present state of knowledge of cooperative processes derives to no small extent from the theory of helix-coil transitions developed to explain the phenomena observed in polyamino-acids. One of the most absorbing of these has always been polyproline, partly because it provides a unique example of a conformational change arising from an isomerization of the peptide bond, and partly because it represents the most basic model for collagen.

Polyproline exists in two ordered states: form I is a right-handed helix in which the peptide bands are in the *cis*-configuration, and form II, a left-handed helix with *trans* peptide bonds. According to solvent and temperature, slow transitions can be made to occur between these two states, the mechanism of which has been a matter of conjecture. The most illuminating study on this subject comes now from Torchia and Bovey (*Macromolecules*, **4**, 246; 1971), who have used high-resolution proton magnetic resonance (220 MHz) to follow what happens when polyproline I is dissolved in water. Peaks caused by the protons on the α -carbon atoms in the *cis* and *trans* forms can be distinguished, the former being split into two components, one of them corresponding to only one proton per polymer chain of twenty-five residues. As the isomerization proceeds, the major *cis* proton resonance progressively gives place to one in the *trans* position, whereas the smaller one remains almost constant until the reaction is largely complete. The fraction of *cis* form can be determined from the areas under the peaks, and its conversion follows zero-order kinetics.

These observations are sufficient to define a mechanism for the transition: in the first place inspection of the structural formula of the repeating unit shows that a *trans* α -carbon proton will see essentially the same environment whether its neighbour on the N-terminal side is itself *cis* or *trans*. By contrast a *cis* residue on the C-terminal side brings the two adjoining α -carbons into a different relationship than when it is a *trans* residue. Thus the junction between two runs of *cis* and *trans* residues will give rise to a unique *trans* resonance from the residue at the junction, only if the *cis* block is on its C-terminal side. The converse is true for the *cis* proton signals. On the evidence of the zero-order kinetics, the single

unique *cis* proton per chain throughout the transition, and the absence of splitting in the *trans* resonances, it then seems to follow that the isomerization goes stepwise, one residue at a time, from the C-terminal end.

In addition to this pleasing syllogism, Torchia and Bovey have been able to clarify the nature of the structural collapse that has long been known to occur at high concentrations of some salts. Two explanations had previously been advanced: an increase in the range of permitted backbone torsional angles, so as to allow deviation from the geometry defining the helix, without isomerization of the *trans* peptide bonds, or the introduction of *cis* bonds at intervals. The absence of line-sharpening, which would accompany increases in freedom, and the appearance of the characteristic *cis*-resonance demonstrates that the second explanation is the correct one. The unfavourable nature of *cis-trans* junctions is thought to be overcome by the ability of *trans-cis-trans* or *trans-cis-cis* constellations of peptide carbonyls to bind cations.

Mattice and Mandelkern (*J. Amer. Chem. Soc.*, **93**, 1769; 1971) have examined the viscosity of polyproline II in water as a function of molecular weight. The intrinsic viscosity can be related to a function that characterizes the rigidity of the molecules in solu-

tion. In high concentrations of salts, such as calcium chloride, the values of the function reflect the collapse of the stiff, extended structure that Mattice and Mandelkern infer to be present in water solution, and they show, in terms of the theory relating molecular dimensions with torsional angles in the backbone, that the introduction of only 5–10 per cent of *cis* peptide bonds would be sufficient to account for the changes.

The arguments concerning the nature of the transition between forms I and II, it should be noted, relate only to aqueous solution, and it seems that a very different situation obtains in other solvents. In a series of solvent systems studied by Winklmair, Engel and Ganser (*Biopolymers*, **10**, 721; 1971), first-order kinetics are observed. An analysis of the kinetics, as observed by a relaxation method, involving perturbation of the system by changing the solvent composition (analogous to the temperature-jump method), leads to a remarkably good fit of the results. The transition in these solvent systems is strongly cooperative, with a very slow rate of nucleation (that is to say formation of *cis-trans* junctions), compared with propagation. These results constitute an interesting test of the theory of conformational transitions, developed in its most refined and general form by Schwarz.

Inhibitory Role of Catecholamines in Ganglia

IN next Wednesday's *Nature New Biology*, de Groat and Saum present new evidence for an inhibitory function of catecholamines in ganglionic transmission. The authors have studied ganglionic transmission in the parasympathetic ganglia on the surface of the cat urinary bladder, using electrophysiological methods, and have recorded the responses evoked in postganglionic fibres by stimulation of the preganglionic pelvic nerve. Simultaneous stimulation of the hypogastric nerve—the sympathetic input to the bladder—inhibited ganglionic transmission in the parasympathetic ganglion on the ipsilateral side. This inhibitory effect of sympathetic stimulation could be blocked by an alpha adrenergic blocking drug, dihydroergotamine, and the inhibitory effect of sympathetic stimulation was mimicked by close arterial injection of small amounts of adrenaline, noradrenaline or dopamine. Histochemical studies had previously revealed that catecholamine-containing nerve terminals make intimate contact with the parasympathetic ganglionic neurones (Norberg and Sjöqvist, *Pharmac. Rev.*, **18**, 743; 1966), suggesting therefore that the sympathetic input to the urinary bladder may inhibit the tissue at least in part by blocking ganglionic transmission in the excitatory

parasympathetic nervous system.

The presence of catecholamine-containing nerve terminals has also been shown in many other autonomic ganglia, of both the parasympathetic and sympathetic nervous systems. Furthermore, there have been various descriptions of the existence of small adrenergic interneurons, or chromaffin-like cells in various ganglia of both divisions of the autonomic system (Norberg and Hamberger, *Acta Physiol. Scand.*, Suppl. 238; 1964; Jacobowitz, *J. Pharmacol. Exp. Ther.*, **158**, 227; 1967). According to a recent report, such small cells in mammalian sympathetic ganglia contain the catecholamine dopamine, rather than noradrenaline which is present in the postganglionic neurones of the sympathetic system (Bjorklund *et al.*, *Acta Physiol. Scand.*, **78**, 334; 1970), suggesting that in addition to the well known role of noradrenaline at neuroeffector junctions of the sympathetic nervous system, catecholamines may also be involved as inhibitory transmitters modulating ganglionic transmission in the autonomic nervous system. Such inhibitory effects may be mediated either by nerve terminals of sympathetic postganglionic neurones, or in some cases may involve small adrenergic inhibitory interneurons within the ganglia.