PROTEIN CONFORMATION

Return of the Native

from our Molecular Biology Correspondent

THE pathways by which unfolded protein chains regain their native, folded state have many traps, which can retard or even prevent completion of the process. These traps, or subsidiary minima of conformational energy, may involve, for example, incorrect disulphide pairings, or aggregated forms. It might then be supposed that any agencies that bring about a stabilization of the native conformation would favour the return to that state, just as they would militate against any departure from it.

Thus the native protein is envisaged as in a state of dynamic equilibrium with other, less organized, conformational variants, the equilibrium concentration of which is well below the threshold of detection. Nevertheless, such processes as exchange of the less accessible hydrogens must be presumed to proceed through such an equilibrium, and likewise the digestion of native proteins by proteolytic enzymes. In the presence of a substrate or inhibitor, which binds only to the native state, and therefore stabilizes it with respect to the denatured state, the inhibition of hydrogen exchange and proteolysis is to be anticipated. A good example can be found in the work of Anfinsen and his colleagues on staphylococcal nuclease. In a recent study, for example (Taniuchi *et al.*, *J. Biol. Chem.*, **244**, 4600; 1969), they have shown to what a remarkable extent the enzyme is stabilized by a specific inhibitor, deoxythymidine diphosphate, together with calcium ions, towards hydrolysis by three different proteases. After an incubation leading to almost complete loss of activity of the uncomplexed enzyme, there is essentially no damage when the nucleotide and calcium are present.

The effect of an appropriate ligand on the renaturation of an unfolded chain has not hitherto been so clearly demonstrated. A striking case has recently been described by Andersson, however (*Arch. Biochem.*, **133**, 277; 1969). Whereas the native state of many proteins that contain disulphide bonds has been recovered from the fully reduced unfolded form, no success seems ever to have been recorded with proteins, such as the albumins, which have many such bonds—seventeen in the case of bovine serum albumin. It has even been suggested that the information content in such a case may be insufficient to direct the return of the chain to a unique native state. With bovine serum albumin, Andersson has found, as have others, that reoxidation yields a product which contains polymers, and which has none of the physical properties of the native protein. It is well known, however, that the protein has an unusual affinity for various ligands, most notably the long-chain fatty acids, which bind very strongly to the native molecule. In the presence of caprylate or palmitate, atmospheric oxidation yields a product with less polymeric material, and a monomer fraction identical in terms of a series of physical characteristics with the pristine protein. These include the *p*H-solubility profiles, optical rotation, tryptophan fluorescence spectrum, which is a sensitive index of the environment of this residue, and some ligand binding isotherms.

Because the protein has no measurable biological activity, identity with the native state cannot in the last resort be proved. Moreover, as work from Foster's laboratory has shown, the native conformation seems to make possible certain variations in disulphide pairings, so that again it is less precisely defined than that of the familiar small enzymes. Nevertheless, the results of refolding of albumin in the absence of ligands clearly give a quite different and disordered product, and Andersson's results seem to show the first recognizable renaturation achieved with a protein of such complexity.

GEOTROPISM

New Light on Statoliths

from our Plant Physiology Correspondent

A MUTANT strain of corn with much smaller starch grains than normal has been used to test a seventy year old explanation of the way plants detect the direction of gravity and grow towards it. Apparently Haberlandt was right in 1900 when he suggested that the large starchy plastids, found in many plant tissues which respond to gravity, initiate the response. These plastids, or starch statoliths, usually rest on the membrane lining the lowest wall of the cell. If the orientation of the cell is disturbed, the statoliths sediment through the cytoplasm until they come to rest on the membrane lining the new lowest wall. This contact between wall and starch grain is believed to constitute the primary geotropic stimulus, and to trigger the growth processes which result in geotropic curvature.

Because gravity can influence the plant cell only by acting on one or more of its component parts, the statolith hypothesis has seemed quite reasonable, and no feasible alternative has yet been suggested. There are obstacles to the complete acceptance of the idea, however. Pickard and Thimann objected that some plants have starch statoliths, but do not respond to gravity. They tested the significance of their objection by removing the starch grains from wheat coleoptiles (the sheaths around the primary leaves). They did this by incubating the tissues at 30° C in a mixture of kinetin and gibberellic acid. After this the coleoptiles understandably grew more slowly than if incubated in sucrose alone, but they still responded to gravity. This geotropic sensitivity in coleoptiles devoid of statolith starch seemed to be good evidence against the statolith hypothesis.

But results which go some way towards tipping the balance in favour of statoliths have been provided recently by Hertel, de la Fuente and Leopold (*Planta*, **88**, 204; 1969), using the new mutant corn. They have compared the movement of statolith starch grains in normal corn and in the mutant with its particularly small starch grains. They found that in the mutant, starch grains moved much more slowly through the cell than they did in normal corn, and that if the coleoptiles were turned from a vertical to a horizontal position, a smaller percentage of starch grains moved downwards into the lower half of the cell in the mutant. Similarly the speed and extent of geotropic growth curvature were proportionally smaller in the mutant corn.

Apart from this laggardly geotropic curvature, the growth of the mutant corn seemed normal, and in particular the transport of the growth regulator indolyl-3-acetic acid (IAA) from the tip of the coleop-