NUCLEOHISTONES

Histones and DNA

from our Cell Biology Correspondent

In the past two years, histone chemistry seems, even to the most dyed in the wool sceptics, to have regained respectability. Better techniques of separation have reduced the seeming chaos of histone fractionation to something like reproducible order; the discovery that histones can be acetylated and phosphorylated has suggested a possible mechanism for the derepression of DNA templates repressed by histones; and Smith and his collaborators have proved that the amino-acid sequences of the same species of histone from organisms as unrelated as peas and cows differ only by two amino-acids out of 102. The argument goes that if, during the evolution from the common ancestor of peas and cows, a protein sequence has been retained virtually unchanged, protein must be fulfilling a crucially important role.

But in spite of all this activity, the precise structure of DNA histone complexes is still obscure. Does, for example, the histone fit into the larger of the two grooves along the DNA helix as Wilkins and his colleagues suggested years ago or to some other part of the DNA molecule? Olins (*J. Mol. Biol.*, **43**, 439; 1969) has just reported a series of physico-chemical measurements of complexes of DNA with lysine-rich histone which support the idea that the histone binds to the large groove of DNA. He finds, as others have, that the arginine and lysine residues of histone (f1 from calf thymus) effectively neutralize DNA phosphate groups. The DNA remains in the *B* form when associated with the histone and can therefore still bind actinomycin, and the f1 histone itself, when complexed with DNA, has little or no helical structure. Evidence that the f1 histone is in the large groove of DNA comes from experiments with complexes of the histone and T2 DNA. T2 DNA can be glucosylated and the glucose residues of the mono and di-glucosylated 5-OH methylcytosine residues of the DNA lie in the large groove. Olins therefore tested f1 to see if it would reduce the level of glucosylation of T2 DNA, presumably by competing for space in the large groove, and found that it does. He proposes two models of f1 nucleohistone. In the first model, the non-basic residues of the histone are looped away from the DNA, enabling the basic residues to neutralize successive phosphate groups; in the second model, one histone is stretched along the length of about twelve turns of the DNA helix, a segment in which about seventy-five per cent of the DNA phosphate groups would not be neutralized by histone basic residues and are therefore free for interaction with other cations.

In an attempt to define more clearly the interaction of DNA phosphate groups with basic amino-acids, Shapiro, Leng and Felsenfeld (*Biochemistry*, **8**, 3219; 1969) have followed the binding of polylysine to DNA as a function of polymer chain length and solvent conditions. They find the binding stoichiometric, cooperative and unexplainably selective for AT-rich regions of the DNA. In the same issue of *Biochemistry* (page 3214), Brutlag, Schlehuber and Bonner report a technique which may be useful for separating nucleohistone complexes on the basis of their buoyant density. When nucleohistones are treated with formaldehyde the histone molecules become cross-linked to the DNA, which means they can be banded in caesium chloride gradients without dissociating.

ANAEMIA

Red Cell Enzyme

from our Medical Biochemistry Correspondent

One type of non-spherocytic haemolytic anaemia is now known to be caused by the presence of an abnormal glutathione reductase in the red blood cell. This condition is particularly interesting because, although glutathione (GSH) has been known for a long time, its precise functions are still not clear. Staal, Hellemann, de Wael and Veeger (*Biochim. Biophys. Acta*, **185**, 63; 1969) have recently purified the abnormal GSH reductase from a patient with haemolytic anaemia caused by this abnormality and have compared its properties with those of GSH reductase from normal human erythrocytes (Staal, Visser and Veeger, *Biochim. Biophys. Acta*, **185**, 39; 1969; Staal and Veeger, *Biochim. Biophys. Acta* **185**, 49; 1969).

It had been claimed that the abnormal enzyme has different kinetic properties from normal, but the work was criticized because the differences found were not great and could have been within the range of experimental error. Staal and Veeger found that the reactions of the normal enzyme with the two nicotinamide coenzymes were very different. The maximum velocity of the reaction with reduced nicotinamide adenine dinucleotide phosphate (NADPH) was affected by the sodium ion concentration, while the reaction with reduced nicotinamide adenine dinucleotide (NADH) was not. $NADP^+$ inhibited the NADPH reaction, which was also inhibited by high concentrations of oxidized glutathione (GSSG) and NADPH, but the reaction with NADH did not show similar effects.

The activity of the abnormal enzyme changed in the same way with a change in the concentration of sodium ions and had the same *p*H optimum as the normal enzyme. The K_m values for GSSG and NADPH were also the same as for the normal enzyme. Unlike earlier workers, Staal *et al.* could not demonstrate any significant differences between the two enzymes with respect to the catalysis of glutathione reduction. There was, however, a big difference in the K_{ass} determined for the reaction of the abnormal apoenzyme with its flavine adenine dinucleotide (FAD) prosthetic group. The activity of the holoenzyme (apoenzyme with FAD) was reduced if it was incubated with the similar but inactive flavin mononucleotide (FMN). Addition of FAD to the abnormal enzyme preparation made it more stable, for the enzyme was protected against heating and the loss of activity during purification was reduced. All these factors suggested that the chief difference between the normal and abnormal enzymes lay in the binding of the FAD prosthetic group. The abnormal erythrocyte may contain the same amount of apoenzyme, but much less active holoenzyme than normal erythrocytes. This suggested that it might be possible to treat this condition by giving relatively large quantities of flavine. In this particular patient, administration of FMN for a few weeks increased the activity of GSH reductase in the erythrocytes almost to normal and activity declined when the treatment was stopped—a good example of biochemical investigations leading to rational therapy.