PROTEINS

Sighting the Sites

from our Molecular Biology Correspondent

A STRONG contender for this year's *concours d'élégance* of protein chemistry is to be found coyly nestling in the yellow pages of the current issue of the *Biochemical Journal*. This is an article by Green, Konieczny, Toms and Valentine (**125**, 781; 1971), and though it is overtly about the binding of biotin to avidin, both the principles and the results are of much wider relevance.

The background is as follows: avidin is a protein made up of four identical chains, of some 15,000 molecular weight. Each of these has one binding site for biotin, with which it combines with enormous avidity. The only part of the biotin molecule indispensable for binding is the ureido ring. By analogy with what is known about the binding of ligands to enzymes, it might be supposed that the binding site would be situated in a cleft or pit in the surface of the protein. It should be possible, Green and his colleagues reasoned, to find out something about the nature of this cleft, and also about the disposition of the subunits in the tetramer by looking at the interaction of avidin with bifunctional ligands.

Green *et al.* therefore synthesized a series of *bis*biotin derivatives, in which the rings were joined by hydrocarbon chains of different lengths, such that the number of bonds between the biotin carboxyl groups varied between nine and twenty-five. Below some critical chain length it would be supposed that the separation of the biotins would be too short to reach between binding sites on two protein molecules if these extend well below the surface, and indeed it was found that when the number of bonds was anything less than twelve, the bifunctional reagent was able to react with only a monofunctional stoichiometry, and no associated forms of the protein could be detected. At twelve, bifunctional behaviour set in, as judged by the combining ratios and also by the appearance of polymers in the electron microscope. These polymers took the form of linear chains of width corresponding to that of single avidin molecules. The shape of the ligand titration curves (determined by displacement of a dye from the binding site), as well as the ease with which the aggregates could be depolymerized by addition of an excess of ligand, indicated, however, that these chain lengths were barely sufficient for stable cross-linking. The polymers showed few branch points, and being linear, evidently consisted of protein molecules joined by way of two bifunctional ligands to either neighbour. A periodicity corresponding to the width of the avidin tetramer was clearly visible. When the chain length was increased above fourteen bonds, the polymers became more stable, and clear daylight could be discerned between successive units. At very high ligand chain lengths, the polymers became much shorter, betokening the formation of intramolecular links by the *bis*biotin compound.

From these results much of interest can be deduced. The minimum length between the biotin carboxyls that will enable both ends to seat in the active site cavity is 15 Å. Above 17.5 Å the binding strength at both ends becomes comparable with that of the monofunctional ligand. It may thus be deduced that the carboxyl group at the extremity of the ligand locates some 9 Å below the surface of the protein. Moreover it follows from the progressive increase in periodicity of the polymer with ligand chain length, and also of the binding constant within the critical range of chain lengths, that the surface is in some degree compressible. The compression is evidently energetically expensive, and becomes exorbitant below a chain length of twelve bonds. (In long chains, the hydrocarbon, judged by the periodicity, is by no means extended, but obviously increases the thickness of the layers between the protein units.) The length of hydrocarbon at which intramolecular cross-linking sets in also gives a limiting measure of the separation of the binding sites on two subunits within an avidin tetramer. Taking 9 Å as the depth of the carboxyl group, it turns out that the sites must be separated by only 6–10 Å on the surfaces of the subunits. This, of course, could be increased if there were any kind of depression or channel between the sites, to a maximum of about 25 Å.

Contemplation of the possible arrangements of equivalent subunits within a tetramer reveals that they may be related by two-fold or four-fold symmetry (isologous or heterologous association). Only the former can lead to linear polymers, one molecule thick. The four-fold arrangements can lead to two-dimensional networks, or to a unidimensionally repeating system, in which, however, each molecule is associated in zigzag manner to two others. Thus although the subunits are too small to be resolved within the tetramer by electron microscopy, the electron microscope nevertheless discriminates between the possibilities, and establishes the symmetry relation between the subunits, showing them to be arranged in two pairs, tail-to-tail, with a two-fold axis down the middle, and a screw axis relating successive repeating units in the chain. The known accessibility of the bound biotin to biotin-requiring enzymes suggests that it lies in a rather broad cleft, partly exposed, very much like inhibitors in enzymes of known structure.

A fashionable protein, the subunit structure of which confers on it its characteristic biological properties, is concanavalin. The crystal structure is being hotly pursued in two laboratories, and Becker, Reeke and Edelman (*J. Biol. Chem.*, **246**, 6123; 1971) have now deduced from an electron density map of a complex of concanavalin with heavy atom containing ligands the disposition of the saccharide binding sites—arguably the most interesting feature that is to be had from the structure. There are four subunits, each of which possesses one saccharide binding site. The 2.8 Å electron density map shows that

Lead Ions in the Atmosphere

THE topical subject of cluster ions is aired in next Monday's *Nature Physical Science*, where Castleman and Tang of the Atmospheric Chemistry Research Group at Brookhaven National Laboratory describe the clustering of water molecules on ions of lead. The possible role of ion clusters in the upper atmosphere is receiving increasing attention, so that the laboratory demonstration by Castleman and Tang of the existence of clusters involving lead may soon find an application in theoretical studies on the transport of lead, both radioactive and stable, in the atmosphere. Their data may also account for the low mobility of radioactive lead ions in humid atmospheres.

Briefly, Castleman and Tang passed an ion beam consisting of Pb^+ through a reaction cell in which the concentration of water vapour could be altered, and the products were examined by mass spectrometry. Ions of the form $Pb^+(H_2O)_n$, where n can be as high as seven, were detected. Castleman and Tang find three peaks in the mass spectrometric output for each cluster, corresponding to the major lead isotopes of mass 206, 207 and 208, and the peak corresponding to mass 204 can also be detected.

As well as demonstrating that clustering on lead ions can occur, the experiment has given some of the parameters of the reactions involved. From these data Castleman and Tang are already able to draw some conclusions relevant to the behaviour of lead in the atmosphere. For example, they say that lead ions in the troposphere resulting from the decay of radon will initially exist as clusters, and the species $Pb^+(H_2O)_6$ should be the most abundant. Such cluster ions will be removed by interaction with aerosols or by recombination with negative ions in the atmosphere.