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Concluding Remarks

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Concluding remarks

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At the conclusion of the Royal Society discussion on lysozyme a little over two years ago, I had to follow Monod and elevate myoglobin and haemoglobin to the rank of honorary enzymes so as to draw my generalizations from three structures rather than a single one. I then suggested that the powerful catalytic activity of enzymes was due to the medium of low dielectric constant provided by their non-polar interior, which allowed strong electrical interactions to take place between the active site and the substrate.

The structures of the six additional enzymes which have since been solved have confirmed this view, but they have also shown that it should be broadened. It appears that in the medium of low dielectric constant a free charge introduces a large free energy. To regain stability two alternative devices are open to the enzyme: (1) to compensate the charge internally; (2) to design a system of bonds which transfers the charge to the surface.

The first principle is used in the activation of the serine proteinases and the second in the construction of their active sites. The second is a new principle, not predicted by any theory of proteolytic catalysis, which will clearly transform current thinking about the catalytic mechanism. It appears to be common to the serine proteinases of the chymotrypsin type as well as those of the subtilisin type and may well apply also to the SH proteinases.

On the assumption that the asparagine seen to be linked to His-159 in the active site of papain might, on further examination, turn out to be an aspartic acid, and in the light of some of the remarks made in the discussion, the active sites of the serine and SH proteinases might be constructed on similar principles. However, at present the electron density map suggests that the distance between the imidazole ring and the SH group is larger than would be expected if they were linked by a hydrogen bond.

The activation of the serine oxygen to a powerful nucleophile by the buried negative charge of the aspartic acid explains why certain analogues of the active site, constructed by organic chemists on what appeared to be reasonable assumptions, nevertheless had a catalytic activity lower than chymotrypsin by a factor of a thousand. These analogues contained the hydroxyl and imidazole groups in sterically favourable positions, but lacked the buried negative charge now seen to be required to activate them.

The specificity site appears to have the function of drawing the substrate so close to the active centre that water is excluded. The relation between the active sites of chymotrypsin and trypsin is particularly interesting. They both consist of similarly constructed, deep pockets close to the active site. The former has a hydrophobic lining attractive to the side chains of tyrptophan, phenylalanine and tyrosine, while the latter carries the negative charge of an aspartic acid at its base, designed to attract the side chains of lysine and arginine. Papain, is the first proteolytic enzyme shown to have a specificity site (for phenylalanine) one residue removed from the peptide bond to be split, which suggests that a search for subsidiary specificity sites in other proteolytic enzymes might be rewarding.

What determines the complex conformation of these proteins? In chains of the myoglobin and haemoglobin type, the determining feature consists of 30-odd sites which have to carry

non-polar side chains, but their exact nature does not seem to matter too much, at least not as far as conformation is concerned. In the serine proteinases, on the other hand, the internal sites seem to be mostly restricted to one specific residue. This suggests that many of them may be required not only to determine the conformation of the enzyme but also to ensure its function, by playing some subtle part in conformational changes which may accompany the binding and splitting of substrate.

The numbers of different proteins in *Escherichia coli* is of the order of a few thousand and in mammals perhaps of the order of a few million. On that basis, our efforts could make only a minor contribution to the understanding of living systems. However, it has looked for some time as though the number of *types of enzymes* would prove to be much smaller than the total number of enzymes, the structure of many enzymes with quite different catalytic properties being variations on a common theme. We should soon begin to classify enzymes on a structural basis and think about the minimal number of protoenzymes, or protogenes, required by a living system.

In conclusion I should like to thank the contributors for the beautiful new forms of Nature which they have revealed to us.

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