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General Discussion

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General discussion

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I would like to return to the main theme of this meeting and ask the theoretical chemists who are here whether they think that the degrees of specificity that have been mentioned during the meeting can be explained by specific binding in terms of the kinds of intermolecular forces discussed yesterday by Professor Buckingham, or whether in certain cases it is necessary to postulate something beyond specific binding. I have in mind Dr Blow's question yesterday, when he referred to the small effect on binding that would be expected to result from the *absence* of a methyl group, and the point that Dr Dalziel made today about the failure of NADP dehydrogenases to work with NAD, which differs only by the *absence* of a phosphate group.

K. Dalziel (*Department of Biochemistry, Oxford*)

In reply to Professor Huxley's question about the NAD and NADP specificity of dehydrogenases, my point was only that the reason why NADP-specific enzymes do not react with NAD is less obvious than the opposite, where we can see, from the structures of the NAD or ADPR complexes, that a bulky phosphate group on the 2′-hydroxyl of the adenine ribose would cause steric hindrance, as well as preventing hydrogen bond formation to aspartate. With an NADP-specific enzyme, the absence of this phosphate in NAD would prevent a salt-linkage, which might be a major contribution to coenzyme binding, or the phosphate might be vital for an induced-fit response by the protein. But as I said earlier, we must await the structure of an NADP complex.

Dr F. H. C. Crick, F.R.S. (*M.R.C. Laboratory for Molecular Biology, Hills Road, Cambridge*)

The answer to Huxley's question is that, so far, it has not been found necessary to postulate unknown intermolecular forces to explain the high degree of specificity often found in biological systems. In those cases in which the specificity appears to be higher than one might expect, a detailed examination of the process involved has shown that this extra specificity is obtained by doing the job in a different way from the straightforward one.

The first case he mentions is a good example of this. The amino acid isoleucine has one more methyl group than the amino acid valine. Both those amino acids are common components of almost all proteins and it is obviously important that in the process of protein synthesis the cell should distinguish between these to a high degree of accuracy. The experimental error rate, of putting valine in positions where isoleucine should go, was carefully investigated by Loftfield some time ago. He found to his surprise that he could detect no errors at all within the sensitivity of his method, which was about 1 part in 3000. The expected error rate for a valine going into an isoleucine 'cavity' was of the order at 1 in 10 or 1 in 50, very far from the experimental limit.

Now although the smaller amino acid (valine), can fit into a cavity designed for the bigger one (isoleucine) with only a small loss of binding energy the converse is far from true. Due to the rapid increase of the repulsive forces at short distances, described by Professor Buckingham,

it takes a lot of energy to cram an isoleucine residue into the smaller cavity needed for a valine. I therefore suggested to Loftfield, during the discussions of his paper in Cambridge, that it would be found that the latter process would be the basis for the very high precision he had found experimentally. This prediction was subsequently confirmed by Berg and his colleagues. They showed that although valine could be bound to some extent to the activating enzyme for isoleucine, when the enzyme attempted to transfer the valine to the isoleucine tRNA this incorrect amino acid was instead hydrolysed off by the enzyme. Presumably in the case of the correct amino acid (isoleucine), its greater size prevented this step. In short, natural selection had invented an editing step which, because it can use the high discrimination of the repulsive forces, permits a high level of overall specificity.

This is not the only case when an editing step is used to improve accuracy. Orgel and I deduced on theoretical grounds that such a step was necessary in DNA replication and suggested to Professor Kornberg that his DNA polymerase enzyme would have such an activity, which he and his colleagues showed to be the case.

When we look at biological mechanisms we are seeing the end result of a long process of natural selection. If nature cannot get high specificity by the direct process she will evolve a gimmick to achieve her aims. This is easier than attempting to change the laws of quantum mechanics.

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