Dr T. L. Benjamin (Public Health Research Institute, New York), for example, described mutants of polyoma virus which only replicate in 3T3 cells transformed by polyoma. Untransformed 3T3 cells, which are permissive hosts of wild type polyoma, and hamster BHK cells, a non-permissive host of wild type virus, are stimulated to undergo DNA synthesis and gain T antigen when infected by these mutant viruses. But these cells are never abortively or stably transformed by the mutants, and the cell surface changes associated with transformation, the exposure of sites which bind wheat germ lipase, are not detectable. Does this mean that the crucial event in transformation is a change in the properties of the cell membrane? Ultimately it should be possible to define the genomes of the small DNA tumour viruses by isolating mutants of each gene, but from the reports at Houston there is a long way to go yet.

In vitro transformation of cells with carcinogenic chemicals has by contrast progressed rapidly, as delegates in this field never missed an opportunity of noting. Dr C. Heidelberger's work at the University of Wisconsin, in particular poses interesting questions. He can transform single C3H mouse cells, a permanent aneuploid line of prostate cells, with methylcholanthrene. The transformed cells show no evidence of RNA or DNA tumour viruses and they seem to have unique antigens. Fourteen clones derived from transformed single cells were tested in pairs for antigenic cross reactivity and none was found. As Heidelberger noted, it may in this system be possible to study carcinogenesis and mutagenesis of the same molecule in the same cell.

ENZYMES

Sites and Subsites

from our Molecular Biology Correspondent

THE creation of a catalytic site in an inactive precursor molecule by proteolytic cleavage of one or more peptide bonds is a widely distributed phenomenon. It occurs in all, or nearly all, of the more familiar proteases, and seems invariably to involve fission near the N-terminus. This, it has been pointed out, is not surprising, for otherwise biosynthesis, which proceeds from the N-terminal end, might result in premature unleashing of proteolytic activity. Just how cleavage of a peptide bond causes the active site to become operational has been a principal objective of the crystallographic studies of Kraut and his group on chymotrypsinogen. Some new details of the structure at 2·5 Å, and its comparison with the chymotrypsin model, determined by Blow and his colleagues, are now given by Freer *et al.* (*Biochemistry*, **9**, 1997; 1970).

The comparison is with α-chymotrypsin, which is formed by further proteolysis of several intermediates, the first of which, π-chymotrypsin, is already fully active, and has only one break in the chain—between arg-15 and ile-16. Crystallographic studies all show that, excision of two dipeptides aside, the structures of α and π-chymotrypsins are identical. The first and most obvious feature of the comparison of enzyme with zymogen is that they are overall very similar, but differences can be discerned at several points. The new α-amino group of ile-16 turns inwards, by virtue of a backbone rotation of 180°, to make an ion-pair with an interior carboxylate, asp-194. The side chains of ile-16 and val-17 are folded into the interior; asp-145 and met-192 at the same time swing outwards into the solvent. There is also a movement of the asp-194 side chain, which breaks a hydrogen bond to his-40 in order to make the ion-pair. This in turn leaves the his-40 free to rotate so as to make a new bond to a backbone carbonyl. The juxtaposition of the vital catalytic residues, ser-195, his-57 and asp-102, is, however, almost undisturbed during activation. There are minuscule changes around his-57, and a small movement at ser-214, a residue which is surmised to be in some way involved in the binding of substrate. A more obvious change concerns the cavity, in which aromatic side chains of the substrate evidently seat. This is not present in the zymogen, and is created by the displacement of met-192.

The moral which the authors draw from all this is that though one may fancy one can define the catalytic mechanism of chymotrypsin, a deeper understanding is lacking. Why does the almost perfectly aligned active site not function in chymotrypsinogen? Is it because of the small adjustments in the position of his-57? Or because the cavity is occluded? And why indeed is a cavity necessary, if elastase, which is so similar, can function without one? The crystallographers' reach, it is owned, still exceeds their grasp.

The finer points of design in even small monomeric enzymes go beyond the definition of the catalytic site. Many cases have now been recognized of additional binding sites that direct the specificity of hydrolytic enzymes, and it is likely that these are a perfectly general feature. In lysozyme the existence of binding sites for acetylglucosamine residues, other than the catalytic centre, was deduced from the crystallography of the complexes. A new study by microcalorimetry (Bjurulf *et al.*, *Europ. J. Biochem.*, **14**, 47; 1970) of the binding of the monomer, dimer and trimer reinforces this with direct measurements of the thermodynamic characteristics of the catalytic site and two subsites.

An impressive treatment of subsites in the amylase of *B. subtilis*, which repays close study, has also just appeared (Thoma, Brothers and Spradlin, *Biochemistry*, **9**, 1768; 1970). A series of oligomeric substrates, up to twelve glucose units in length and labelled at the end-group, was prepared, and the distribution of the lengths of the labelled products of the enzymatic hydrolysis was determined by chromatography. The results were analysed with the assumption of an independent free energy contribution for binding at each subsite, so that the values govern the partition of any oligomer between possible runs of adjacent sites. This leads to an evaluation of the relative free energies of association of identical monomer units at nine measurable sites, the catalytic centre being between positions six and seven. The tenth position is grossly unfavourable —it is assumed in consequence of steric hindrance— and this explains immediately the hitherto mysterious propensity of the enzyme to cleave near the end of short substrate chains, because oligomers bind in such a way as to avoid this position, and may therefore abut on it, but will not in general extend into or beyond it. The relative free energies of binding that are derived give remarkable internal agreement with the proportions of the various products. The approach, and the results, are clearly of the widest interest in enzymology.

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