PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 485th Meeting of the Society was held at University College, Cork, on Thursday and Friday, 12 and 13 September 1968, when the following papers were presented.

COLLOQUIUM ON 'HYDROLYTIC ENZYMES'

Primary Structures of Serine Proteinases

By B. S. HARTLEY. (*Medical Research Council Laboratory of Molecular Biology, Cambridge*)

The sequence homologies between the pancreatic serine proteinases (Hartley, Brown, Kauffman & Smillie, 1965; Neurath, Walsh & Winter, 1967) appear in a new light after the elucidation of the tertiary structure of α -chymotrypsin A. In this model (Sigler, Blow, Matthews & Henderson, 1968), about two-thirds of the side-chains can be described as 'surface' and the rest as 'buried' or 'internal'. All of the charged groups except Asp-194 and the N-terminus Ile-16, and all but two of the asparagine and glutamine residues, lie in the surface. Of the large hydrophobic residues, half lie in the interior and half in the surface. The 'histidine loop' (residues 42-58), the 'serine loop' (residues 191-220) and the 'methionine loop' (residues 168-184), which are regions of high sequence homology in these proteinases, thread their way through the centre of the molecule and are held by the four disulphide bridges common to the whole group.

Chymotrypsin B is almost identical to chymotrypsin A in activity, but has 51 sequence differences (Smillie, Furka, Nagabhushan, Stevenson & Parkes, 1968). Almost all of these are in 'surface' residues and the remaining ten differences in 'internal' side-chains are very conservative chemically. In trypsin, only 30% of the 'surface' residues are identical, but 70% of the 'internal' residues are homologous with chymotrypsin A and the remaining changes are generally very conservative: there are only three cases where hydrophilic side-chains replace 'internal' hydrophobic groups of chymotrypsin. Moreover, the 14 'deletions' in trypsin relative to chymotrypsin occur at positions in the chain that lie in the 'surface'. The partial sequence results for porcine elastase (D. M. Shotton and B. S. Hartley, unpublished work) tell a similar story. The overall homology with chymotrypsin A is 42%, but 58% of the internal residues are identical and most of the internal changes are very conservative. Therefore the large hydrophobic side-chains that occupy most of the interior of the chymotrypsin molecule have very similar counterparts in trypsin and elastase,

and one can conclude that the tertiary structures are probably very similar indeed. This conclusion is elegantly illustrated by the observation that the two disulphide bridges of trypsin that are not homologous with chymotrypsin can be fitted to the model without disturbing the polypeptide chain (Sigler *et al.* 1968).

Do these homologies extend to other serine proteinases? Our sequence studies of bovine thrombin (synthesized in the liver) indicate that it has two chains of 49 and about 270 residues, and has three disulphide bridges. The A-chain sequence (S. Magnusson, E. Merler, J. Wootton and B. S. Hartley, unpublished work) shows no homology with the pancreatic enzymes, and it may be a large 'activation peptide' linked to the B chain by a disulphide bridge, as in chymotrypsin. We have determined the sequence of tryptic and peptic peptides from the B chain of thrombin that account for 220 residues, and we can already see considerable homology with chymotrypsin in the N-terminus (residues 16-22 of chymotrypsin), the 'methionine loop' region (residues 176-185) and the active-centre serine region (residues 191-202) (S. Magnusson and B. S. Hartley, unpublished work). The disulphide bridge at position 201 is, however, replaced by a methionine residue in thrombin. It seems likely that thrombin will also prove similar to chymotrypsin in tertiary structure, and that the mechanism of activation of prothrombin will be similar to that of chymotrypsinogen.

Biochemists will indeed be grateful if they are allowed to assume that enzymes of different specificities but similar catalytic mechanism will prove to have homologous sequences and similar tertiary structures. Pepsin and rennin may be another such class of proteinase (Foltmann & Hartley, 1968), but a word of caution is necessary. Subtilisin is a serine proteinase with an apparently similar mechanism to chymotrypsin, but has no detectable sequence homology (Smith *et al.* 1966). Convergent evolution may yet be found on the molecular level.

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Chymotrypsin: Tertiary Structure and Enzymic Activity

By D. M. BLOW. (*Medical Research Council Laboratory of Molecular Biology, Cambridge*)

In addition to the electron-density map of α -chymotrypsin at high resolution (Sigler, Blow, Matthews & Henderson, 1968) electron-density maps have been prepared at lower resolution for chymotrypsinogen (Kraut, Sieker, High & Freer, 1962), and for π -, δ - and γ -chymotrypsins (Kraut, Wright, Kellerman & Freer, 1967; Matthews, Cohen, Silverton, Braxton & Davies, 1968). The process leading from the zymogen to the various forms of the enzyme involves no major refolding, and the activation step appears to depend on the insertion of the α -amino group of Ile-16 into an interior position in the molecule where it can interact with the β -carboxylate group of Asp-194. It is proposed that when Asp-194 is drawn into the interior, the active-centre residues, including His-57 and Ser-195, are brought into the correct stereochemical relationship.

The crystals have been shown to exhibit specific chymotryptic activity, though at reduced rates, despite the fact that the crystals are only stable up to about pH 6.3. In attempts to discover the mode of binding of the substrate, the binding positions of *N*-formyl-*p*-iodophenylalanine and of *N*-formyl-phenylalanine have been studied in three dimensions. A detailed study has been made of the differences of structure between the native enzyme and the tosylated enzyme.

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Mechanisms of Enzyme-Catalysed Hydrolysis Reactions: Present Status and Outstanding Problems

By H. GUTFREUND. (*Department of Biochemistry, University of Bristol*)

The exciting developments in the three-dimensional structure analysis of enzyme molecules

have confronted the enzyme mechanic with reality. In the case of some enzymes, the combination of suitable substrates with techniques that permit the resolution and characterization of individual enzyme-substrate compounds, the approach of modern chemical kinetics has proved itself dominant in the elucidation of mechanisms and in the design of meaningful crystallographic and chemical experiments. In other cases, such as lysozyme (Blake *et al.* 1967) and carboxypeptidase (Reeke *et al.* 1967), studies of the reactions in solutions gave only limited results before the concrete information obtained from crystallographic studies. In these latter systems, the structural data are the ones that show the way to the design of meaningful chemical and kinetic experiments.

Here I am mainly concerned with enzyme systems that have been fruitfully studied by the methods of chemical kinetics. The hydrolytic enzymes that fall into this group are those that have either a specially reactive serine hydroxyl group or a cysteine thiol group at the active site. These two large groups of enzymes probably have a number of important common features. One must be aware of the dangers of generalizations, but one should not forget the special importance of phenomena that are found to occur widely. I shall discuss only three enzymes of 'serine active site' group in any detail and we shall try to learn our lessons from these: chymotrypsin, trypsin, and alkaline phosphatase from *Escherichia coli*.

One important lesson that can be learned from studies of these three enzymes is that the use of over-simplified methods can provide a great deal of invaluable information, but becomes dangerously misleading if not rapidly abandoned at the right time. I am referring to the use of pseudo-substrates and the extensive interpretation of steady-state kinetics. The first of these is probably the major problem of the future. We must obtain information about the interaction of the natural substrates with hydrolytic enzymes. The specific conformation and reactivity of the enzyme-substrate complex is surely dependent on the specific substrate used. Yet nobody will deny that we have learned a lot about hydrolytic enzymes from studies with pseudo-substrates, and even with reagents that bear no structural relation to the substrate. We are, however, at the point of making dangerous extrapolations from these studies. This major problem of substrate specificity in the widest sense will benefit most from complementary studies between crystallography and chemical kinetics.

In the area of reaction mechanisms for the three enzymes mentioned, we have been faced with the problem that one of the principal arguments involved in the characterization of acyl and phosphoryl enzyme intermediates by steady-state