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Chymotrypsin: Tertiary Structure and Enzymic Activity

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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

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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