kinetics is wrong, is still widely used and leads to quantitative results that are misleading. This opens up the second major problem to be solved in detail, which is closely related to the first one: the response of the enzyme to interaction with their specific substrates. The problem can be most clearly stated by a discussion of the hydrolysis catalysed by *E. coli* phosphatase. Phosphate esters with widely different leaving groups are hydrolysed at identical rates. Phosphoryl enzyme can be shown to occur as a reaction intermediate, and the obvious explanation for the identical reaction rates was the hypothesis that the hydrolysis of the intermediate was rate-determining in the case of all the substrates. Rapid-flow techniques have enabled us (Trentham & Gutfreund, 1968; Fernley & Walker, 1966; Aldridge, Barman & Gutfreund, 1964) to show that at optimum pH it is, in fact, the phosphorylation of the enzyme that is rate-determining and identical with a range of substrates. This indicates that there is some step between the Michaelis complex and the phosphoryl enzyme common to the reaction of different substrates, which is independent of the leaving group. The response of the enzyme to form the reactive intermediate could provide some explanation for this.

With trypsin and chymotrypsin, a series of

experiments with chromophoric substrates led us to the conclusion that these enzymes too formed another significant intermediate between the Michaelis complex and formation of the acyl enzyme (Barman & Gutfreund, 1966; Bernhard, 1968). These findings will be elaborated in relation to the exploration of the reactivity of the active site. The fact that during amide and peptide hydrolyses, none of the enzyme intermediates, except the initial Michaelis complex, occur at a significant concentration has to be borne in mind. Only carbonyl derivatives of specific amino acids with good leaving groups have so far permitted the detection of other intermediates.

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COMMUNICATIONS

The Protection of Peptides from Biological Sources for Use in the Chemical Synthesis of Proteins

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A common approach to the chemical synthesis of polypeptide chains involves starting with amino acids having protected side-chain functional groups, and producing protected oligopeptides by stepwise condensation. These larger fragments are subsequently coupled to produce the final sequence.

Since many such projects involve the production of sequences close to those found in nature, we have investigated the protection of tryptic peptides of proteins for use in place of synthetic ones. The peptides were purified from digests of hen's-egg lysozyme, initially in batches of tens of milligrammes. Since most normal methods of protection were developed for free amino acids, it was not always clear whether they would cause peptide-bond cleavage or other side reactions in peptides, or even if they would work at all. The benzylation

method of Ciperia & Nicholls (1955), for example, cleaved labile peptides. However, benzylation with phenyldiazomethane (Sarin & Fasman, 1964) in ethanol-water (5:1, v/v) at pH 4.5 (cf. diazomethane: Chibnall & Rees, 1958) gave seemingly quantitative conversion of the peptides studied into products with properties consistent with the benzylation their carboxyl function.

If protection is possible, it is necessary to free the terminal carboxyl and amino groups (to allow coupling to proceed) without unblocking the side-chain groups (which must remain protected throughout). This can be done if protection is applied to the whole protein before tryptic digestion, and it is this method that is being developed for side-chain amino protection (to be published). Although preliminary experiments show that this may be done for carboxyl groups also, it has been found easier to protect them at the tryptic-peptide stage. The terminal carboxyl only may be removed very rapidly by trypsin (in one case roughly 50%/min. at 37° with 1% of enzyme by weight) which is an esterase (Schwert, Neurath, Kaufman & Snoke, 1949; Kloss & Schröder, 1964) bound to cleave groups in such a position. It was essential to find a

solvent (pyridine–water, 1:1, v/v) for protected peptides in which trypsin remained active.

Work is continuing with the further chemical characterization of the protected peptides and on problems presented by coupling. It is proposed to investigate the use of other enzymes and reagents for protection and deprotection.

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Enquiries concerning patentable applications of this work should be made to the National Research Development Council.

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On the Allosteric-Effector Specificity of Trypsin Substrates

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Activation of trypsin by both substrate and product is well known (Trowbridge, Krehbiel & Laskowski, 1963; Béchet & Yon, 1964; Howard & Mehl, 1965; Trenholm, Spomer & Wootton, 1966; Gemperli, Hofmann & Rottenberg, 1967; Labouesse & Gervais, 1967), but this effect is almost exclusively restricted to derivatives of toluene-*p*-sulphonyl-L-arginine.

We are now testing a number of related compounds for their ability to act as allosteric effectors on trypsin. The results will be discussed in terms of a model, trypsin being taken as a unique example of a single chain protein subject to allosteric activation.

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The Substrate Specificity of Pepsin C

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Pepsin C is an acid protease from the stomach of the pig which hydrolyses haemoglobin but which has little activity against acetyl-L-phenylalanyl-L-di-iodotyrosine (APD). The latter is hydrolysed by pepsin at pH2 at a rate of about 10 moles/mole of enzyme/min. Pepsin C hydrolyses the B chain of oxidized insulin and at pH2 the initial rate of hydrolysis is about 80 bonds/mole of enzyme/min. The major sites of attack were reported as the Ala-14–Leu-15 and Tyr-16–Leu-17 bonds (Ryle & Porter, 1959). The acetyl and benzyloxycarbonyl derivatives of dipeptides containing these bonds and benzyloxycarbonyl-L-histidyl-L-alanyl-L-leucyl methyl ester (cf. Inouye, Voynick, Delpierre & Fruton, 1966) were tested at pH2, and were hydrolysed by pepsin C at less than 1% of the rate of peptic hydrolysis of APD. The last substrate, which is less insoluble than the others, was hydrolysed somewhat faster at pH4.

The sites of attack of pepsin C on the B chain of oxidized insulin were re-investigated by using a two-dimensional separation of the peptides and quantitative analysis of their amino acid composition. From the compositions of the peptides found and the known amino acid sequence of the substrate, the major sites of hydrolysis were identified (percentage hydrolysis in parentheses): Tyr-16–Leu-17 (86%); Phe-24–Phe-25 (82%); His-10–Leu-11 (46%); Ala-14–Leu-15 (44%); Glu-13–Ala-14 (34%). These results are in general agreement with those obtained earlier except that the extent of hydrolysis of the Phe–Phe bond appears to have been underestimated.

To test the possibility that rapid hydrolysis of these bonds by pepsin C only occurs when they are found in a longer peptide, some hexapeptide derivatives related to the sequence of the B chain of oxidized insulin were tested as substrates. The hexapeptides were synthesized by the solid-phase method (Merrifield, 1965). Benzyloxycarbonyl-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-(*S*-benzyl)-L-cysteinylamide was hydrolysed, the initial rate being about 2.7 bonds/mole of enzyme/min.

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