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 pH 2 at a rate of about 10 moles/mole of enzyme/min. Pepsin C hydrolyses the B chain of oxidized insulin and at pH 2 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. Inoué, Voynick, Delpierre & Fruton, 1966) were tested at pH 2, 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 pH 4.

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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