Resistance of a Cyclic Peptide to Enzymic Attack

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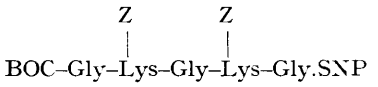
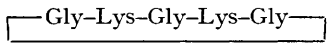
CYCLIC peptides of the tyrocidine class resist enzymic attack,[1] and similarly the side chain but not the cyclic nucleus of polymixin B_1 is degraded by subtilisin.[2] However, the structures of these bacterial polypeptides are unusual, including D-amino-acids, and the cause of resistance to enzymes is obscure. We now report a case where a cyclic peptide is resistant to trypsin, although it complies with the thoroughly established structural requirements for substrates of that enzyme.

The *p*-nitrophenyl thiolester (I)[3] of a protected



(I)

pentapeptide was prepared *via* the corresponding methyl ester from α-t-butyloxycarbonyl-ε-benzyloxycarbonyl-L-lysine[4] by standard methods[5] avoiding racemisation, the final stage being reaction with tri-*p*-nitrophenyl phosphorotrithioite in dimethylformamide.[6] The t-butyloxycarbonyl group was selectively removed by hydrogen chloride in ethyl acetate and then cyclisation[7] was achieved in 52% yield by treatment of a dilute solution in dimethylformamide with Amberlite IRA-410 (OH^- form). The benzyloxycarbonyl groups were removed by hydrogen bromide in acetic acid. The dihydrobromide was added to a column of IRA-410 (OH^-) to remove open-chain compounds, and the resulting free base (II) was converted into the amorphous dihydrochloride dihydrate. The monomeric nature of this cyclic peptide was shown by dinitrophenylation, which afforded *only* monodinitrophenyl derivatives (not separated by electrophoresis at pH 6·1) and fully dinitrophenylated peptide in addition to unchanged cyclic peptide.[8]



(II)

The cyclic pentapeptide (II) was completely resistant to trypsin during 24 hr., under conditions which cleaved open-chain H–Gly–L–Lys–Gly–L–Lys–Gly–OH fully in 3 hr. Indeed this was a useful preparative method for isolation of the cyclic peptide from polypeptides, which were by-products

[1] R. D. Hotchkiss, *J. Biol. Chem.*, 1941, **141**, 184; *Adv. Enzymol.*, 1946, **4**, 169.
[2] T. Suzuki, K. Hayashi, K. Fujikawa, and K. Tsukamoto, *J. Biochem. (Japan)*, 1963, **54**, 555.
[3] Symbols according to R. Schwyzer, J. Rudinger, E. Wünsch, and G. T. Young in "Peptides," ed. G. T. Young, Pergamon Press, Oxford, 1963, pp. 262—269.
[4] R. Schwyzer, A. Costopanagiotis, and P. Sieber, *Helv. Chim. Acta*, 1963, **46**, 884.
[5] Dicyclohexylcarbodi-imide was the condensing agent except in one instance. Satisfactory C, H, and N analyses were obtained at all stages.
[6] J. A. Farrington, P. J. Hextall, G. W. Kenner, and J. M. Turner, *J. Chem. Soc.*, 1957, 1407.
[7] G. W. Kenner, P. J. Thomson, and J. M. Turner, *J. Chem. Soc.*, 1958, 4148.
[8] *Cf.* A. R. Battersby and L. C. Craig, *J. Amer. Chem. Soc.*, 1951, **73**, 1887; 1952, **74**, 4023.

when cyclisation was carried out in aqueous dimethylformamide buffered with magnesium carbonate. Experiments with mixed substrates confirmed that the cyclic peptide did not appreciably inhibit the action of trypsin. The cyclic peptide was also unaffected by subtilisin, which degraded the open-chain pentapeptide.

Resistance to enzymic attack must be ascribed to the inability of the cyclic pentapeptide to assume the necessary conformation in the enzyme–substrate complex, although the ring is large enough for all the amide groups to be *trans*. Obviously this would become possible in a very large cyclic peptide, and the location of critical ring size is an intriguing question.

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