Inter-chain Aminolysis, a Novel Side-reaction in Solid-phase Peptide Synthesis

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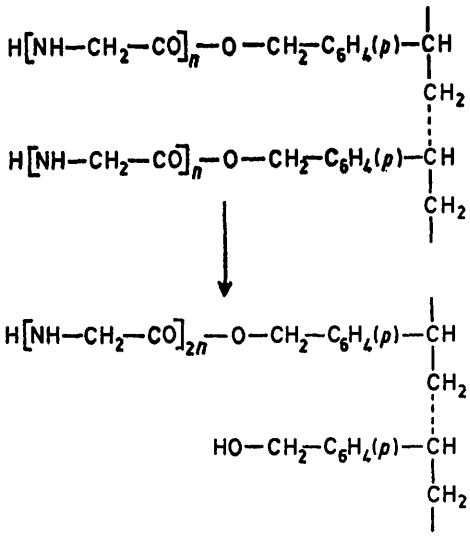
Summary A novel side-reaction in solid-phase peptide synthesis leading to chain doubling, by inter-chain aminolysis, is reported.

SOLID-PHASE synthesis has proved to be quite useful.[1] There is, however, difference of opinion on the length of a purifiable peptide which can be synthesized; according to the originator[2] a protein of 124 residues or according to some authors only 12 or 15 residues at the most.[3] It is obvious that a knowledge of side-reactions is of the greatest importance for this. We report a previously unknown side-reaction, *viz.* inter-chain aminolysis.

In the procedure according to Merrifield the acidic cleavage of the *N*-protecting group is followed by "neutralization", *viz.* an excess of 10% (v/v) triethylamine in methylene chloride.[1] In this step, aminolysis of the peptide–polymer bond by the free amino-group of another polymer-bound peptide is possible; the result, therefore, is chain



SCHEME. *Inter-chain aminolysis in solid-phase peptide synthesis*

doubling (Scheme). We observed this phenomenon during the neutralization of glycine-, diglycine-, triglycine-, and tetraglycine-on-resin. Di-, tetra-, hexa-, and octaglycine, respectively, were therefore formed on the polymer. The doubled chains will give rise to failure sequences even when all other reactions go to completion and to reactive hydroxymethyl groups.

N-t-Butyloxycarbonyl-diglycine polymer (Boc-di-Gly-P) was prepared by esterification of Boc-Gly-Gly with a chloromethylated styrene–2%-divinylbenzene polymer (P). Tri-Gly-P and tetra-Gly-P were prepared by coupling of Boc-Gly and Boc-Gly-Gly, respectively, to this polymer. On removal of Boc-groups and neutralization the peptides were cleaved from the polystyrene by HBr/trifluoroacetic acid.[1]

The formation of doubled peptides was demonstrated with the aid of an automated amino-acid analyser, with which glycine and di- to octa-glycine, inclusive, could be completely separated. For this, use was made of a cation exchange resin (Spherix XX-8-60-0) and a citrate buffer (pH 3·35, 0·2 M-Na^+). The order of elution is glycine, octaglycine, heptaglycine, hexaglycine, pentaglycine, tetraglycine, diglycine, and triglycine. This order was accounted for by two separation mechanisms, ion exchange and gel permeation.[4] By calibrating the amino-acid analyser it was also possible to perform quantitative measurements.

TABLE

Peptide inter-chain aminolysis in 10% (v/v) triethylamine–methylene chloride

Starting material	Reaction time 10 min	Reaction time 20 h	Product
Glycine-P	—	trace	Diglycine
Diglycine-P	1%	1%	Tetraglycine
Triglycine-P	trace	5%	Hexaglycine
Tetraglycine-P ..	—	3%	Octaglycine

In the normal neutralization time (10 min) a maximum of 1% of tetraglycine was formed from diglycine-P. For triglycine P the percentage was found to increase to 5% after 20 h. We observed no chain doubling when the neutralization was omitted.

In the case of the dipeptides-on-polymer the dioxopiperazine formation,[5] an intra-chain aminolysis, will probably play a more important role than the interchain aminolysis. We expect that with activated resins, *e.g.*, nitrated resins,[6] the inter-chain aminolysis will occur to a higher degree and that it may be avoided by the use of aminobenzhydryl resins.[7] The degree to which the inter-chain aminolysis occurs, in our case favoured by the sterically unhindered Gly–Gly bond, will be determined, *inter alia* by the load of the resin. We used 0·5 to 0·7 mmole of peptide/g of peptide resin. Furthermore, the degree of cross-linking may be important.

The results are listed in Table 1.

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[1] R. B. Merrifield, *J. Amer. Chem. Soc.*, 1963, **85**, 2149; *Adv. Enzymol.*, 1969, **32**, 221; A. Marglin and R. B. Merrifield, *Ann. Rev. Biochem.*, 1970, **39**, 841.
[2] B. Gutte and R. B. Merrifield, *J. Biol. Chem.*, 1971, **246**, 1922.
[3] R. Geiger, *Angew Chem.*, 1971, **83**, 155; E. Wünsch, *ibid.*, 773.
[4] H. A. Billiet (Laboratory of Analytical Chemistry, Technische Hogeschool, Delft), to be published *in Chromatographia.*
[5] M. Rothe and J. Manzanek, *Angew. Chem.*, 1972, **84**, 290; B. F. Gisin and R. B. Merrifield, *J. Amer. Chem. Soc.*, 1972, **94**, 3102.
[6] H. Takashima, V. du Vigneaud, and R. B. Merrifield, *J. Amer. Chem. Soc.*, 1968, **90**, 1323.
[7] P. G. Pietta and G. R. Marshall, *Chem. Comm.*, 1970, 650.