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Notes

Peptide Synthesis by Fragment Condensation on a Soluble Polymer Support. 8.[1] Maximum Peptide Chain Lengths of Carboxyl Component Peptides for Effective Coupling Reactions with Amino Component Peptides Anchored to Soluble and Cross-Linked Polystyrene Supports

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Peptide synthesis by fragment condensation on a polymer support has been thought to be one of the most promising methods for synthesizing large peptides and proteins.[2] However, one serious problem of the method is, as often observed, its low yields in fragment condensation reactions on cross-linked resin supports. It is believed that the problem results from the restricted permeability of carboxyl component peptides into resin matrices.[2-4] In addition, it has been demonstrated that a 1%-cross-linked polystyrene support commonly used in solid-phase peptide synthesis is sufficiently flexible for interactions among pendant functional groups.[5-7] The onset of a β-sheet aggregation by hydrogen bonding among pendant peptide chains brings about additional cross-linking of the polymer network.[5] In this case, the coupling efficiencies of carboxyl component peptides with pendant peptides were remarkably decreased as the carboxyl component peptide chain is lengthened.[3] On the other hand, we proposed a few strategies for solubility improvement of peptides in which the peptides are forced not to adopt a β-sheet structure.[8-10] As previously proposed,[3] we believe that the combination of these strategies and fragment condensation on a soluble polymer support has promising versatility for syntheses of pure large peptides and proteins.

This paper investigates the effect of peptide chain length of carboxyl component peptides on the coupling yields in the reactions with amino component peptides anchored to soluble polystyrene, and poly(styrene-*co*-1% (and -2%) divinylbenzenes). In the fragment condensation on a polymer support, it is better to use as large a peptide fragment as possible in order to obtain a pure product by simple purification procedures. This study elucidates the maximum chain lengths of carboxyl component peptides for effective coupling reactions with amino component peptides on polymer supports. In the reactions, the carboxyl components should be free from a β-sheet aggregation to evaluate the maximum peptide chain length. All of the carboxyl component peptides used in this study were previously synthesized to ascertain that the concept of "peptide segment separation" is useful as one of the strategies to improve solubility and to show their structures are predominantly in a randomly coiled structure in polar solvents.[9,11]

Experimental Section

Materials. The soluble H-$(\beta\text{-Ala})_2$-*p*-(oxymethyl)phenylacetamidomethylated polystyrene A (amino content, 39 μmol/g) was prepared from Boc-$(\beta\text{-Ala})_2$-*p*-(oxymethyl)phenylacetic acid and aminomethylated polystyrene by the method described previously.[12,13] The soluble H-$(Phe)_3$-*p*-(oxymethyl)phenylacetamidomethylated polystyrene B (amino content, 19 μmol/g) was prepared by copolymerization of styrene (99.6 mol %), Boc-Phe_3-(oxymethyl)phenylacetamidomethylated styrene (0.2 mol %), and divinylbenzene (0.2 mol %) followed by deprotection of the Boc group as described previously.[13] Poly(styrene-*co*-1% (and -2%) divinylbenzene) beads of 200-400 mesh, Bio-Beads S-X1 and S-X2, were purchased from Bio-Rad Laboratories. They were aminomethylated as described in the literature,[12] coupled with Boc-$(\beta\text{-Ala})_3$-*p*-(oxymethyl)phenylacetic acid and deprotected to give cross-linked H-$(\beta\text{-Ala})_3$-*p*-(oxymethyl)phenylacetamidomethylated polystyrene C (cross-linked with 1% divinylbenzene) and D (cross-linked with 2% divinylbenzene), respectively.

The large carboxyl component peptides, Boc-$(Leu_3Pro_2Gly)_n$-OH (n = 1, 2, 4, 6, 8, 10, 12; the peptides 1-7) were those previously prepared.[9]

General Method of Coupling Reactions of Boc-peptides 1-7 with the Peptides on the Polymer Supports A-D. To the solution of each Boc-peptide (10 equiv) in *N*-methylpyrrolidone (NMP) or in NMP/CH_2Cl_2 (volume ratio 1/1) (1 mL), each of the amino component peptide polymers A, C, or D (100 mg) was added. Then, HOBt (10 equiv) in the solvent (0.5 mL) and DCC (10 equiv) in the solvent (0.5 mL) were added to the reaction mixture, and it was stirred for 2 days at room temperature. When using the soluble peptide polymer A, the reaction mixture was poured into ethanol (20 mL) with stirring. The precipitated peptide polymer was filtered off, washed with ethanol, and dried in vacuo at 50 °C. When using the cross-linked peptide polymer C or D, the mixture was filtered off and the residue was washed with ethanol and dried in vacuo at 50 °C. Each resulting peptide polymer was subjected to acid hydrolysis and then to amino acid analysis. Coupling yield was obtained from the ratio of each corresponding peptide content and the β-Ala content of the peptide polymer.

When using the peptide polymer B, 4 equiv each of Boc-peptide, HOBt, and DCC was used and coupling reactions were carried out in NMP for 5 days at room temperature.

Results and Discussion

It has already been established that the carboxyl component peptides 1-7 used here have a randomly coiled structure in highly polar solvents such as NMP,[9,11] and it is also expected that the peptides have a predominantly randomly coiled structure in NMP/CH_2Cl_2. It has also been suggested by NMR studies that the carboxyl component sequential polypeptides have a repeating local conformation characteristic of the internal hexapeptide segment $Pro_2GlyLeu_3$, and the N-terminal Leu_3 and C-terminal Pro_2Gly segments have local conformations common to the sequential polypeptides in polar solvents.[11] Therefore, it is expected that only the effect of peptide

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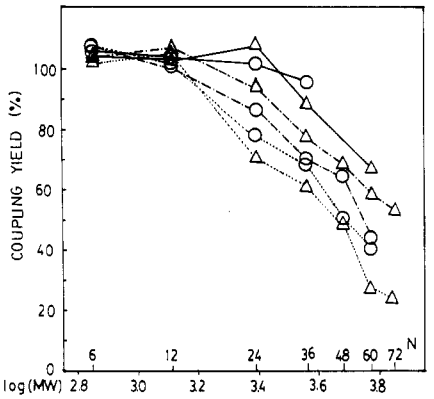

Figure 1. Influence of the peptide chain length of Boc-$(Leu_3Pro_2Gly)_n$-OH on the coupling yield in the reactions with the peptide polymer A in NMP (-△-) and in NMP/CH_2Cl_2 (-○-), with the peptide polymer C in NMP (-·-△-·-) and in NMP/CH_2Cl_2 (-·-○-·-), and with the peptide polymer D in NMP (···△···) and in NMP/CH_2Cl_2 (···○···). MW, molecular weight of the Boc-peptides: N, number of amino acid residues of the Boc-peptides. Coupling yield was obtained from the ratio of each corresponding peptide content (total of Pro, Gly, and Leu contents, devised by the number of amino acid residues contained in the carboxyl component peptide) and β-Ala content of the peptide polymer.

chain length of the carboxyl component peptides on the coupling yield can be detected when the same polymer support is used. It is clear that after these peptides are partially coupled with amino component peptides anchored to polymer supports, pendant peptide chains have no ability to take β-sheet aggregation which brings about additional cross-linking of the polymer network.

Coupling yields of the Boc-peptides 1–7 in the reactions with the peptide polymers A, C, and D are plotted against the value of logarithm of the molecular weights of the Boc-peptides 1–7 (Figure 1). Coupling yields of the large Boc-peptides 4–7 in the reactions with the peptide polymer B are listed in Table I.

In the reactions with the peptide polymer A, Boc-hexapeptide 1 through Boc-tetraeicosapeptide 3 reacted almost quantitatively in both solvents. Boc-hexatriacontapeptide 4 reacted with the peptide polymer A in 90–95% yields and a gradual decrease in the coupling yields was observed in the reactions of the larger carboxyl component peptides of Boc-octatetracontapeptide 5 through Boc-doheptacontapeptide 7 with the peptide polymers A and B (Figure 1 and Table I). The gradual decrease in the coupling yields in these reactions would probably be due to decrease in opportunities to react with the terminal carboxyl groups of the large carboxyl component peptides. Enfolding of the terminal carboxyl groups with their own peptide chains would occur more frequently for the larger peptides and would prevent them from approaching the reaction sites of the peptides anchored to the soluble polystyrenes A and B.

In the reactions with the peptide polymers C and D, Boc-hexapeptide 1 and Boc-dodecapeptide 2 reacted quantitatively. Boc-tetraeicosapeptide 3 reacted with the peptide polymer C in 80–90% yields and with the peptide polymer D in 65–80% yields depending on the kind of solvent used. As in the case of the peptide polymers A and B, a gradual decrease in coupling yields with the increasing peptide chain length of the Boc-peptides was also observed in the reactions with the peptide polymers C and D. The coupling yields of a Boc-peptide (3–7) larger than Boc-dodecapeptide 2 were greater in the reactions with the peptide polymer A and B than in the reactions with the peptide polymer C. The coupling yields in the latter, in turn, were greater than those in the reactions with the peptide polymer D. The decrease in the coupling yields with the increasing cross-linking degree of polymer supports is probably due to the restricted permeability of carboxyl component peptides into resin matrices.[2,3]

Table I
Coupling Yields in the Reactions of Boc-$(Leu_3Pro_2Gly)_n$-OH (n = 6, 8, 10, and 12) with the Peptide Polymer B

Boc-peptide	n	amino acid content, μmol/g				coupling yield,[a] %
		Pro	Gly	Leu	Phe	
4	6	122	58	143	37	79
5	8	106	63	156	32	73
6	10	159	80	207	33	72
7	12	167	79	217	31	64

[a] Coupling yield was obtained from the ratio of Gly and Phe contents.

In the reactions with the peptide polymer C, the coupling yield of a Boc-peptide larger than Boc-dodecapeptide 2 with NMP as the solvent was higher than that with NMP/CH_2Cl_2 as the solvent. In contrast, in the reactions with the peptide polymer D, the higher coupling yield was obtained when NMP/CH_2Cl_2 was used as the solvent. The polystyrene supports appear to be in a more extended form in NMP/CH_2Cl_2 than in NMP, while the Boc-peptides appear to be in a more extended form in NMP than in NMP/CH_2Cl_2. The above results would be due to the counterbalancing effects of the solvents on the polystyrene supports and Boc-peptides.

Registry No. BOC-(Leu_3Pro_2Gly)-OH, 91649-94-4; BOC-$(Leu_3Pro_2Gly)_2$-OH, 91649-95-5; BOC-$(Leu_3Pro_2Gly)_4$-OH, 91649-96-6; BOC-$(Leu_3Pro_2Gly)_6$-OH, 91728-66-4; BOC-$(Leu_3Pro_2Gly)_8$-OH, 91728-80-2; BOC-$(Leu_3Pro_2Gly)_{10}$-OH, 91728-86-8; BOC-$(Leu_3Pro_2Gly)_{12}$-OH, 109333-81-5.

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