CHEMISTRY LETTERS, pp. 749-752, 1989.

Solid Phase Synthesis of Magainin 1 under Continuous Flow Conditions[1)]

Sukekatsu NOZAKI
Faculty of Pharmaceutical Sciences, Josai University,
Sakado, Saitama 350-02

4-(Hydroxymethyl)phenoxymethyl copoly(styrene-1% divinylbenzene) resin beads loosely packed in a glass column was satisfactorily employed as a solid support for synthesis of magainin 1 under Fmoc-strategy. The trieicosapeptide was synthesized in continuously flowing DMF under low pressure conditions. The antibiotic was obtained in an overall yield of 31% after HPLC purification.

Many advantages of the continuous flow method of solid phase peptide synthesis (CF-SPPS)[2-4)] over the traditional batchwise one are known,[5,6)] but the former is still much less popular. The limited application of CF-SPPS in peptide chemistry might be partly due to the recognition that the resin supports commonly used in the batchwise method are troublesome for column operation required in CF-SPPS;[2,6)] the soft resin beads variably swollen in different solvents are often compressed in the column reactor to disturb normal flow of the solvents or reagents and cause unexpected high back pressure. Recently, Krchňák et al. showed in the preparation of some octa- and decapeptide amides that a soft resin, 4-methylbenzhydrylamine copoly(styrene-1% divinylbenzene), is applicable to low pressure CF-SPPS under Boc-strategy, if sufficient space is left in the column packed with the resin.[7)]

Present paper describes that 4-(hydroxymethyl)phenoxymethyl copoly(styrene-1% divinylbenzene), Wang resin,[8)] swollen in DMF can be satisfactorily employed for low pressure CF-SPPS of a trieicosapeptide antibiotic, magainin 1 (Fig. 1),[9,10)] under Fmoc-strategy. The peptide was prepared according to a route illustrated in Fig. 2. N-Fmoc, O-Bu^t-seryl resin (0.32 mmol Ser/g resin, 0.500 g) was placed in a glass column (0.8 x 10 cm) and was swollen in DMF. The resin bed settled in the column was 4.4 cm hight. The column was attached to a manual synthesizer similar to that designed by Dryland and Sheppard.[6)] For removal of Fmoc-group, 20% piperidine in DMF was pumped into the column and then DMF for washing the resin. Acylation was achieved by 2 hours' circulation of a DMF solution of Fmoc-amino acid HOBt

Gly-Ile-Gly-Lys-Phe-Leu-His-Ser-Ala-Gly-Lys-Phe-Gly-Lys-Ala-Phe-Val-Gly-Glu-Ile-Met-Lys-Ser

Fig. 1. Amino acid sequence of magainin 1.

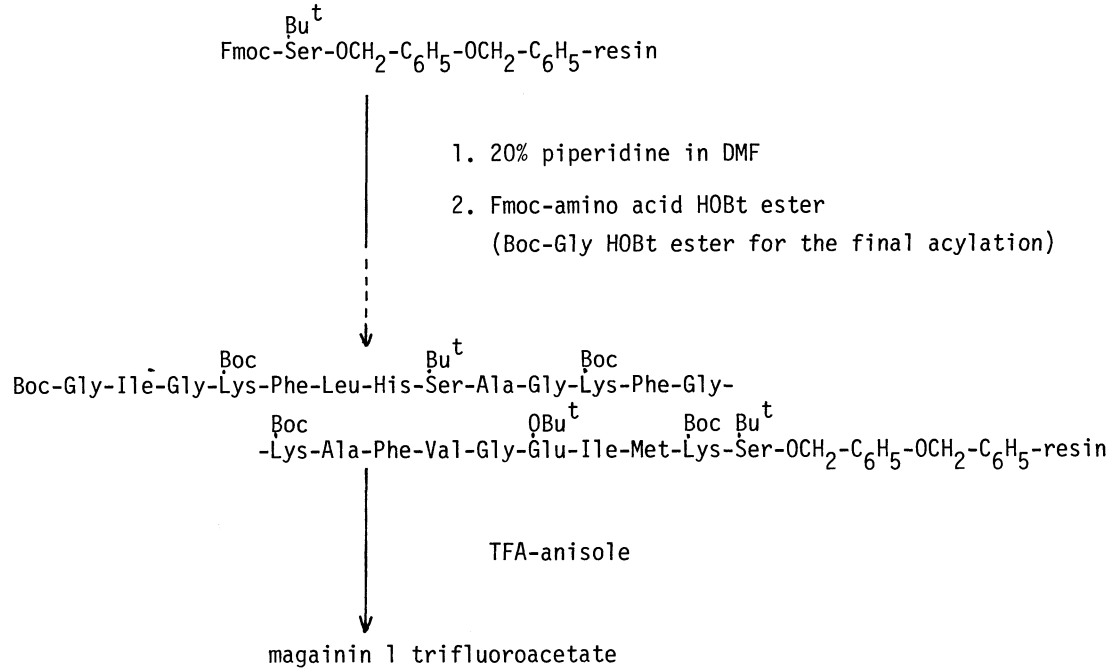

Fig. 2. Synthesis of magainin 1.

ester (3-fold moles per mole of the amine component), which was prepared in a separate vessel by mixing Fmoc-amino acid, HOBt, and DCC in DMF. The excess reagents were then removed from the column by pumping DMF. By repeating the series of the procedures, a protected magainin 1 was synthesized on the resin. Side chain protection was Boc-group for lysine, t-butyl ester for glutamic acid, and t-butyl ether for serine. Histidine was incorporated as the N^{α}-, N^{im}-diFmoc-derivative, and N-terminal glycine as Boc-glycine. The schedule for peptide elongation was summarized in Table 1. Single coupling procedure was employed and no monitoring was carried out. Throughout the synthesis a constant flow of the solvent and reagents at a rate of 2 ml/min was easily maintained by a low pressure ceramic pump without generation of any considerable back pressure (less than 0.3 kg/cm^2 at the final stage of the synthesis). At the end of the synthesis, the peptide-resin bed in the column was 6.7 cm hight indicating during the preparation the volume of the resin bed increased to about 1.5 fold of the original one. The fact is compartible with the data showing that copoly(styrene-1% divinylbenzene) beads expand in the course of peptide elongation.[11] Sufficient space left in a reactor for growing peptides is essentially required for low pressure CF-SPPS on soft resin supports, even when the synthesis is carried out in a combination of solvents similarly swelling the resin.

The crude peptide (Fig. 3-A) cleaved from the resin by TFA-anisole(8:1, 1 h at room temperature) was purified on a semipreparative HPLC column (TSK gel ODS-80TM, i-PrOH/0.1% TFA, 1:2.5) to give highly purified magainin 1 trifluoroacetate (Fig. 3-B) in an overall yield of 31% based on the C-terminal serine attached to the

Table 1. Schedule of Coupling Cycle

Step	Reagent	Mode of liquid delivery[a)]	Time/min
1	20% piperidine in DMF	Flow	10
2	DMF	Flow	20
3	Fmoc-amino acid HOBt ester (3 equivalents) in DMF[b)]	Circulation	120
4[c)]	DMF	Flow[d)]	10

a) A flow rate of 2 ml/min was maintained by a pump. b) Added immediately after removal of dicyclohexylurea by filtration. c) In a separate vessel, 1 M DCC in dioxane was added into a DMF solution of Fmoc-amino acid and HOBt to prepare the active ester used in the next coupling cycle. d) The injector and the circulating line of the synthesizer were rinsed by DMF.

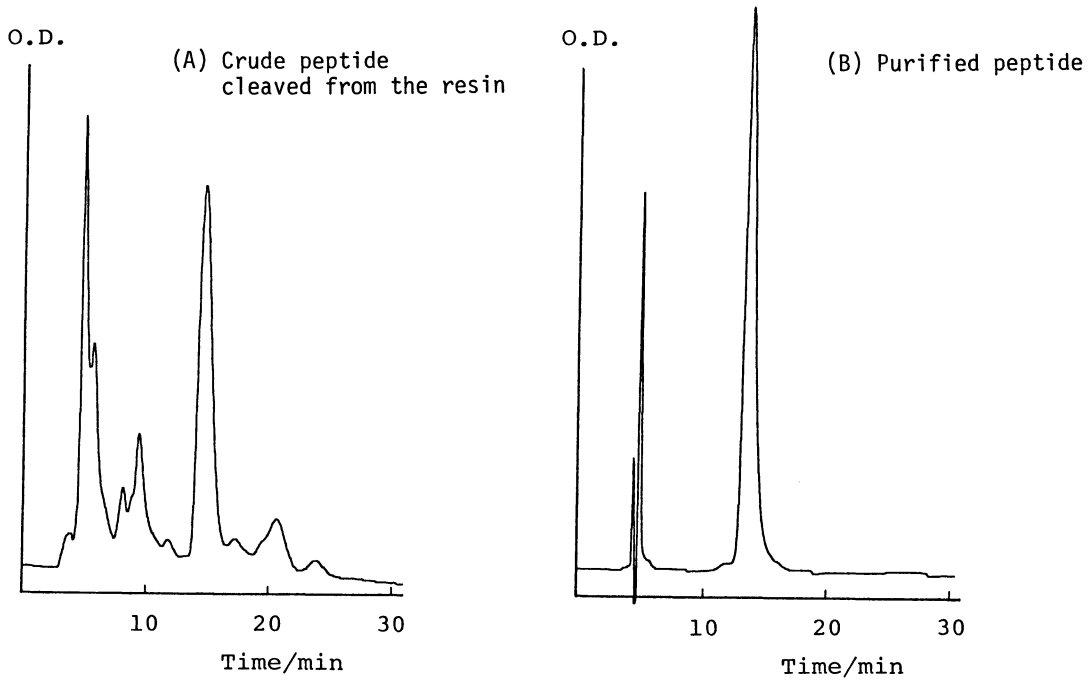

Fig. 3. HPLC profiles of synthetic magainin 1. TSK gel ODS-80TM (0.46 x 15 cm); i-PrOH/0.1% TFA, 1 : 2.5; 0.5 ml/min; 220 nm.

resin. Some properties of the synthetic peptide were as follows. R_f: 0.57 (Avicel cellulose, n-BuOH/pyridine/AcOH/H_2O, 16:10:3:12, ninhydrin detection). Amino acid composition in the acid hydrolysate (6 M HCl, 110 °C, 16 h): Ser 1.70, Glu 1.13, Gly 5.16, Ala 2.22, Val 0.97, Met 0.94, Ile 1.98, Leu 1.07, Phe 2.81, His 1.00, Lys 4.02. $[\alpha]_D^{23}$ -44.8° (c 1, 20% AcOH). Found: C, 45.80; H, 6.21; N, 11.75%. Calcd for $C_{112}H_{177}O_{28}N_{29}S.7CF_3COOH.6H_2O$: C, 45.64; H, 5.96; N, 12.25%. Minimum

inhibitory concentration (μg/ml) towards some microorganisms: 100 (Streptococcus pyogenes 1022), 50 (Streptococcus agalactiae 1020), 50 (Corynebacterium diphtheriae P. W. 8), 50 (Bacillus subtilis ATCC 6633), 100 (Escherichia coli ATCC 25922).

In comparison with the rigid supports specially designed for low pressure CF-SPPS,[3,6] copoly(styrene-1% divinylbenzene) based supports have superior loading capacities of C-terminal amino acids and are less expensive. Present study reveals that low pressure CF-SPPS of a peptide consisting of more than 20 amino acid residues is smoothy achieved in DMF on Wang resin. Low pressure CF-SPPS using the soft resin support would be a convenient technique in peptide synthesis under Fmoc-strategy.

The author is grateful to the staff of the Central Research Laboratory of Toyo Jozo Co. Ltd. for the elemental analyses and the biological assays, and to Prof. Ichiro Muramatsu, Rikkyo University, for helpful discussion.

References

1) Amino acids used are of L-configuration. Following abbreviations were used: Fmoc = 9-fluorenylmethoxycarbonyl, Boc = t-butoxycarbonyl, Bu^t = t-butyl ether, OBu^t = t-butyl ester, DCC = dicyclohexylcarbodiimide, HOBt = 1-hydroxybenzotriazole, TFA = trifluoroacetic acid, DMF = N, N-dimethylformamide.
2) E. Bayer, G. Jung, I. Halász, and I. Sebestian, Tetrahedron Lett., 51, 4503 (1970).
3) R. P. W. Scott, K. K. Chan, P. Kucera, and S. Zolty, J. Chromatogr. Sci., 9, 577 (1971).
4) R. P. W. Scott, S. Zolty, and K. K. Chan, J. Chromatogr. Sci., 10, 384 (1972).
5) T. J. Lukas, M. B. Prystowsky, and B. W. Erickson, Proc. Natl. Acad. Sci. U.S.A., 78, 2791 (1981).
6) A. Dryland and R. C. Sheppard, J. Chem. Soc. Perkin Trans. 1, 1986, 125.
7) V. Krchňák, J. Vágner, M. Flegel, and O. Mach, Tetrahedron Lett., 28, 4469 (1987).
8) S. S. Wang, J. Am. Chem. Soc., 95, 1328 (1973).
9) M. Zasloff, Proc. Natl. Acad. Sci. U.S.A., 84, 5449 (1987).
10) M. Zasloff, B. Martin, and H. C. Chen, Proc. Natl. Acad. Sci. U.S.A., 85, 910 (1988).
11) V. K. Sarin, S. B. H. Kent, and R. B. Merrifield, J. Am. Chem. Soc., 102, 5463 (1980).

(Received February 8, 1989)