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SOLID PHASE SYNTHESIS OF SIGNAL SEQUENCE FRAGMENTS EMPLOYING THE TRANSESTERIFICATION METHOD OF CLEAVAGE

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ABSTRACT

Peptides corresponding to the hydrophobic region of signal sequences have been synthesized by the solid-phase method employing the transesterification method of cleavage from the resin. The protected peptides thus obtained were purified easily by column chromatography on silica gel with varying proportions of methanol and chloroform as eluants.

Most secreted proteins are synthesized as precursors with amino terminal extensions of 15-30 residues[1]. These extensions or 'signal sequences' are characterized by a basic amino-terminal region followed by a stretch of uncharged, mainly hydrophobic amino acids[2]. Interestingly, no primary structure homology exists among signal sequences. Recent studies using recombinant DNA techniques have indicated that signal sequences may have all the information for secretion and proper localization of cellular proteins[3,4]. However, it is still not clear how the specific recognition of signal sequences of varying lengths and primary structure arises. Structural and other physico-chemical studies would aid considerably in understanding the role of signal sequences in the intracellular sorting of proteins. Generation of signal sequences by biochemical methods is not practical as precursor proteins are unstable and difficult to isolate[5]. Synthetic approaches particularly by solid-phase methods have been used to obtain signal peptides[6-8]. However, considerable difficulties, especially in purification of peptides cleaved from insoluable supports by hydrogen fluoride, were experienced[6]. We have explored the transesterification method of cleavage[9] in the synthesis of peptide fragments corresponding to the hydrophobic region of the signal sequence of the E.coli proteins, lipoprotein[10] (peptides 1 and 2) and lambda-receptor[11] (peptide 3) by the solid-phase method.

Boc-Gly-Thr-Thr-Leu-Leu-Ala-Gly-OMe 1

Boc-Gly-Ala-Val-Ile-Leu-Gly-Thr-Thr-Leu-Leu-Ala-Gly-OMe 2

Boc-Thr-Leu-Lys(Z)-Lys(Z)-Leu-Pro-Leu-Ala-Val-Ala-Val-Ala-Ala-Gly-Val-Met-Thr-Ala-Ala-Met-Ala-OMe 3

By this method of cleavage we have obtained fully protected peptides which were conveniently purified by column chromatography on silica gel with varying proportions of chloroform and methanol as eluants.

The first Boc amino acid (the C-terminal amino acid in 1 - 3) was attached to chloromethylated polystyrene-co 1% divinyl benzene resin (200-400 mesh, substitution 1.03 mmol/gm from Sigma Chemical Co. USA) by the cesium salt procedure of Gisin[12]. Solid-phase reactions were carried out on a mechanical shaker in a glass vessel equipped with a fritted disc and a stop-cock.

One cycle of synthesis, based on 1 gm of starting resin, consisted of the following operations : (1) CH_2Cl_2 wash, 15 ml, 3 x 1 min (2) deprotection, 30% or 20% TFA/CH_2Cl_2, 15 ml, 30 min (3) CH_2Cl_2 wash, 15 ml, 3 x 1 min (4) $CHCl_3$ wash, 15 ml, 1 x 1 min (5) prewash 5% triethylamine/CH_2Cl_2, 15 ml, 1 x 1 min (6) neutralization, 5% triethylamine/$CHCl_3$, 15 ml, 10 min (7) $CHCl_3$ wash, 15 ml, 3 x 1 min (9) equilibration with Boc amino acid, 10 min (10) dicylohexylcarbodiimide in 5 ml CH_2Cl_2, 180 min (11) ethanol wash, 10 ml, 3 min. In the synthesis of peptide 3 20% TFA/CH_2Cl_2 was used for deprotection after the addition of Lys(Z) in the presence of 1% dithiothreitol. Progress of the synthesis was monitored by the picric acid test[13].

Cleavage of the peptides from the resin was accomplished by transesterification as follows : A suspension of the peptide-resin (0.500 g) in 30 ml of anhydrous methanol and 4 ml triethylamine was stirred under reflux for 6-8 hours. The resin was filtered and the methanol solution evaporated to yield the crude peptide. Each batch of resin was subjected to three cycles of transesterification to ensure complete recovery of peptides. The recovery of crude peptides was as follows 1, 0.700 g from 2.9 g of resin (2g) yield = 77%; 2, 0.380 g from 1.6 g of resin (1g), yield = 63%; 3, 0.450 g from 2.3 g resin (1.5 g), yield = 56%. Values in brackets indicate amount of starting resin.

Peptides were purified by column chromatography on silica gel. The solvent system used was varying proportions of MeOH/$CHCl_3$. Peptides were checked for homogeneity of tlc on silica gel using the solvent system 10% MeOH/$CHCl_3$ and 15% MeOH/$CHCl_3$. Peptides were visualized by iodine vapor, ninhydrin and starch/KI. The yields of pure peptides were as follows : 1 (5% MeOH/$CHCl_3$) 0.600 g from 0.700 g crude, 2 (10%) MeOH/$CHCl_3$) 0.160 g from 0.380 g crude, 3 (6% MeOH/$CHCl_3$) 0.220 g from 0.450 g crude. The solvent composition

at which the peptides eluted is indicated in brackets. The amino acid composition of the purified peptides was determined after hydrolysis in 6N Hcl in vacuo for 48 hours on an LKB 4151 alpha plus amino acid analyzer. The results of the analyses were as follows :

Peptide 1 Thr 1.95 (2), Gly 1.87 (2), Ala 1.00 (1), Leu 1.90 (2)
Peptide 2 Thr 1.80 (2), Gly 2.96 (3), Ala 1.80 (2), Val 1.00 (1)
Peptide 3 Thr 200 (2), Pro 1.00 (1), Gly 0.85 (1), Ala 7.12 (7), Met 1.90 (1), Val 2.90 (3), Leu 3.10 (3), Lys 1.80 (2)

Theoretical values are indicated in parenthesis. The Boc and Z protecting groups were removed from the purified peptides by neat TFA at 0°C and TFA : m-cresol: thioanisole[14].

Almost all signal sequences have amino acids with short side chains[2] like Gly, Ala or Ser. Hence low yields in the transesterification method of cleavage due to steric hinderance would not be expected in the solid-phase synthesis of these sequences. Also, fully protected peptides would be available for selective labelling for physico-chemical studies. Thus, in the solid phase synthesis of signal peptides or hydrophobic peptides in general, it would be advantageous to use the transesterification method of cleavage.

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