SYNTHESIS OF ELASTIN-LIKE PEPTIDES USING THE LIQUID PHASE METHOD

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Elastin-like peptides were synthesized by the liquid-phase method using poly(ethylene glycol) monomethyl ether (PEGM) (m.w. 5 000) as solubilizing support which provides an alternative possibility for conformational studies and spectroscopic measurements in solution. The following sequences were synthesized: Boc-Ala-Pro-Gly-Val-APEGM, Boc-Ala-Pro-Gly-Val-Gly-Val--APEGM, Boc-Ala-Pro-Gly-Val-Ala-Pro-Gly-Val-Gly-Val-APEGM, Boc-Ala-Pro-Gly-Val-Gly--Val-Ala-Pro-Gly-Val-Gly-Val-APEGM.

Elastin, a highly cross-linked connective-tissue protein, is mainly responsible for elastic mechanical properties of the tissues (*e.g.* aorta, ligament, *etc.*). The interest in the elastic fibres derives not only from efforts to understand mechanically the functioning of the biological elastomers but also from the appreciation that an elastic fiber is a primary site of both classification and lipid deposition in the vascular wall[1-6]. Although the structure–function relationships in several fibrous proteins are fairly clear, elastin is an exception because of its extreme insolubility which is hindering sequence analysis and conformational studies.

In an attempt to understand elastin conformation peptides *I*–*IV* were synthesized by the liquid–phase method[7] to be models of the repeating peptide sequences in tropoelastin, the precursor protein of the elastic fiber of ligaments arterial wall, skin, and lungs[8,9].

I	Boc-Ala-Pro-Gly-Val-APEGM
II	Boc-Ala-Pro-Gly-Val-Gly-Val-APEGM
III	Boc-Ala-Pro-Gly-Val-Ala-Pro-Gly-Val-Gly-Val-APEGM
IV	Boc-Ala-Pro-Gly-Val-Gly-Val-Ala-Pro-Gly-Val-Gly-Val-APEGM

These peptides are bounded to monofunctional poly(ethylene glycol) monomethyl ether (PEGM) of m.w. 5 000, which exerts a strong solubilizing effect on the attached peptide chain in all solvents and provides alternative possibilities for analytical and spectroscopic measurements in solution. It was found also that poly(ethylene glycol)

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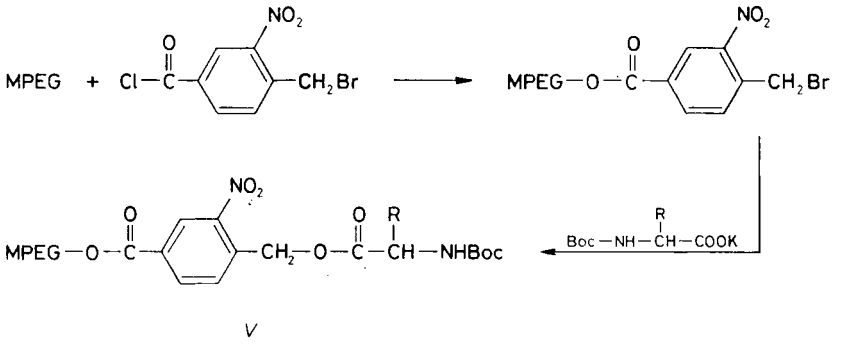
has no influence upon the conformation of the peptide bound to it[10], and commonly used methods, such as IR, UV, NMR, and CD spectroscopy, can be applied directly to peptide poly(ethylene glycol) esters.

To accelerate and simplify the removal of the protected peptide from the resin at the end of the synthesis a photosensitive anchoring group (A) was attached to the poly(ethylene glycol) by esterification of the polymer with 3-nitro-4-bromomethylbenzoyl chloride (ref.[11]) (Scheme 1). The cleavage of the peptide from the soluble polymer proceeded to 98% within 24 h at 350 nm under conditions which neither decompose the aromatic amino acid nor cleave the acid or base-labile protecting groups. The coupling of 3-nitro-4-bromomethylbenzoylpoly(ethylene glycol) monomethyl ether with the first amino acid potassium salt was catalyzed by 18-crown-6-ether. This raised the per cent of incorporation of the tert-butyloxycarbonylvaline into the polymer *V* from 88% to 96·5% (Scheme 1).



V

SCHEME 1

The synthesis of peptides were carried out according to the following cycle: The Boc group was removed by treatment of *V* with trifluoroacetic acid–dichloromethane (1 : 1) for 30 min using 10 ml of the deprotecting agent *per* 1·0 g of the peptide. The volume of the solution was then reduced by flash evaporation to an oil and the poly(ethylene glycol) peptide was precipitated by addition of anhydrous ether under stirring. The mixture was stirred over 15–30 min at 30°C, the precipitate was filtered, washed with ether, and dried under vacuum. The coupling reactions were carried out by symmetrical anhydride method[12] applying excess anhydride component. To this end, the Boc-protected amino acid derivative was dissolved in a minimum amount of dichloromethane and the solution was cooled to 0°C, 0·48 equivalent of dicyclohexylcarbodiimide as a 2 mol l^{-1} solution in dichloromethane was added, and the mixture was allowed to stand 30 min at 0°C. The precipitated N,N'-dicyclohexylurea was removed by filtering the anhydride solution directly into a flask containing the deprotected amino-component in dichloromethane.

The extent of coupling was monitored first by qualitative UV test on thin-layer plates. Quantitative ninhydrin tests[13] were carried out after isolation of protected poly(ethylene glycol) peptide by precipitation. The purity was tested after each step by thin-layer chromatography and amino-acid analysis. All the amino acids were Boc-protected at the α-amino group.

EXPERIMENTAL

All Boc-amino acids were synthesized according to Schnabel's method[14,15].

Boc-Val-OH Potassium Salt

1·0 g of Boc-Val-OH was dissolved in a mixture of ethanol (6 ml), water (4 ml), and equivalent of 1M-KOH solution, the solvent was removed by azeotropic distillation in the presence of toluene and dried *in vacuo* over P_2O_5, the resultant hygroscopic salt was used without further treatment.

Synthesis of 3-Nitro-4-bromomethylbenzoylpoly(ethylene glycol) Monomethyl Ether

3-Nitro-4-bromomethylbenzoic acid (see ref.[1]) was added to thionyl chloride (17·85 g; 0·15 mol). The mixture was heated under reflux for 6 h while stirring. The excess of thionyl chloride was removed by distillation at 80°C. The product was washed with petroleum ether (b.p. 60—80°C), yield 86%. For $C_8H_5BrClNO_3$ (278·6) calculated: 12·74% Cl; found: 12·42% Cl.

60 g (12 mmol) of poly(ethylene glycol) monomethyl ether (m.w. 5 000) and (18 g; 60 mmol) of 3-nitro-4-bromomethylbenzoyl chloride were dissolved in 500 ml toluene and refluxed under nitrogen atmosphere for 24 h. The excess of toluene was distilled under *vacuo* till the total volume becomes 60 ml. The product was precipitated by dropwise addition of ether under cooling and stirring. The product was dissolved in CH_2Cl_2 and precipitated with ether. This step was repeated 3 times till the pure product was obtained. TLC; $R_F = 0$, 1-butanol–acetic acid–water (3 : 1 : 1).

Coupling Reaction of 3-Nitro-4-bromomethylbenzoylpoly(ethylene glycol) Monomethyl Ether with Boc-Val-OH Potassium Salt

A mixture of Boc-Val-OH potassium salt (0·26 g; 1 mmol), MPEGNA (2·. g; 0·5 mmol), and 18-crown-6-ether (0·28 g; 1 mmol) were dissolved in dimethylformamide and refluxed for 48 h. The mixture was then evaporated *in vacuo* and the residue was dissolved in dichloromethane and precipitated with ether under cooling and stirring. The product was filtered off, dried, and dissolved several times in dichloromethane and precipitated with ether till chromatographically pure product was obtained. Incorporation was found to be 96·5% by amino-acid analysis.

Amino-acid analyses. *I*: Ala 1, Pro 0·88, Gly 1, Val 1. *II*: Ala 1, Pro 0·77, Gly 2, Val 1·55. *III*: Ala 2, Pro 1·64, Gly 3, Val 2·35. *IV*: Ala 2, Pro 1·57, Gly 4, Val 3·6.

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