A POLYAMIDE SUPPORT FOR SOLID-PHASE PROTEIN SEQUENCING

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1. Introduction

The solid-phase sequencing procedure of Laursen [1] requires the covalent attachment of a peptide or protein to an inert support. The essential properties of the support are that it should be capable of being highly substituted with functional groups, that these functional groups should be accessible for chemical reaction in a variety of aqueous and organic media, and that it should be stable under the reaction conditions used for the Edman degradation. An ideal support should also be capable of coupling efficiently all sizes of polypeptides including proteins and subsequently allow these to be degraded with a repetitive yield sufficient to obtain at least 30 residues of amino acid sequence.

The present supports – polystyrene [1] and porous glass [2] – do not fulfill all of these criteria. Polystyrene derivatives [1,3] do not swell in aqueous media necessitating the performance of the coupling reactions in solvent systems where the peptide may be poorly soluble. Also, attachment yields for large peptides or proteins are often very low and once attached, these molecules may only be degraded with relatively low efficiency. With porous glass derivatives [2,4] the reverse is true and small peptides are attached only in low yield. The repetitive yield for the solid phase Edman degradation on these supports to be 90–93% [5] allowing only the first 20–25 residues of amino acid sequence to be determined.

A polydimethylacrylamide-based resin has recently been shown [6] to have significant advantages for solid-phase peptide synthesis over conventional polystyrene matrices. Since this resin is already functionalised with (protected) amino groups and appeared to possess all of the essential properties defined above, we have also investigated its use for solid-phase sequencing.

2. Materials and methods

2.1. *Preparation of resin*

The cross linked *t*-butoxycarbonyl(BOC)-β-alanyl-hexamethylenediamine-polydimethylacrylamide resin was prepared essentially as described previously [6], but on a ten-fold scale. On this larger scale, the polymer was obtained in a largely amorphous rather than beaded form. The β-alanine content was 0.24 mmol/g. The terminal BOC-group was removed by subjecting the resin to steps 1–8 of the solid phase synthesis cycle [6] prior to activation of the resin. Dimethylacetamide was used in place of dimethylformamide [6].

2.2. *Activation of resin*

The swollen resin (200 mg) was added in small portions to a gently stirred saturated solution of *p*-phenylenediisothiocyanate (Eastman) in 10 ml dimethylformamide (Pierce, Sequenal grade) over a period of 1 h. Stirring was continued for a further 20 min and the activated resin was then washed on a sintered-glass funnel with dimethylformamide (2 × 20 ml) followed by methanol (6 × 20 ml). The isothiocyanato-polydimethylacrylamide was then dried in vacuo.

2.3. *Attachment of samples*

100 nmol of the B-chain of insulin (Boehringer), native lysozyme (Sigma) and performic acid oxidised [7] basic pancreatic trypsin inhibitor (Bayer) were

each dissolved in 0.4 ml of 0.4 M *N,N'*-dimethylallylamine in 60% (v/v) aqueous pyridine, buffered to pH 9.5 with trifluoroacetic acid. This buffer was purchased ready-made from Pierce. Activated resin (50 mg) was added to each sample and the mixtures gently stirred at room temperature for 30 min. Ethanolamine (50 μl) was then added to block excess functional groups on the support and stirring continued for a further 30 min. The supernatants were then removed and each resin sample washed three times with 3 ml of methanol before drying under water-pump vacuum.

2.4. *Peptide and protein sequencing*

The dry resin, with peptide or protein attached, was mixed with 1 g of glass beads [1] and degraded in a solid-phase sequencer (Sequemat 10K) essentially as described by Laursen [1]. Phenylthiohydantoin (PTH)-derivatives of amino acids were identified by t.l.c. on silica plates [4,8]. Sequencing reagents were obtained from Pierce (Sequenal grade).

3. Results and discussion

The insulin B-chain and lysozyme samples were both degraded through 10 residues. Using the di-isothiocyanate attachment method [9], the N-terminal residue and subsequent lysine residues remain attached to the support. However, the yield of phenylthiohydantoin at step two appeared identical for both the 30-residue B-chain peptide and the 129-residue protein, indicating approximately equal coupling yields in the attachment of both samples to the resin despite their widely different molecular weights. In both cases the coupling yield was distinctly higher than has been found in this laboratory when attaching equivalent amounts of sample to amino-polystyrene [1] and to aminopropyl-glass [2]. Since the t.l.c. method of identification is only semi-quantitative, an accurate value for the degradative efficiency could not be calculated. However, overlap was estimated visually to be less than 5% at step nine and there was only a very faint PTH-amino acid background at step 10

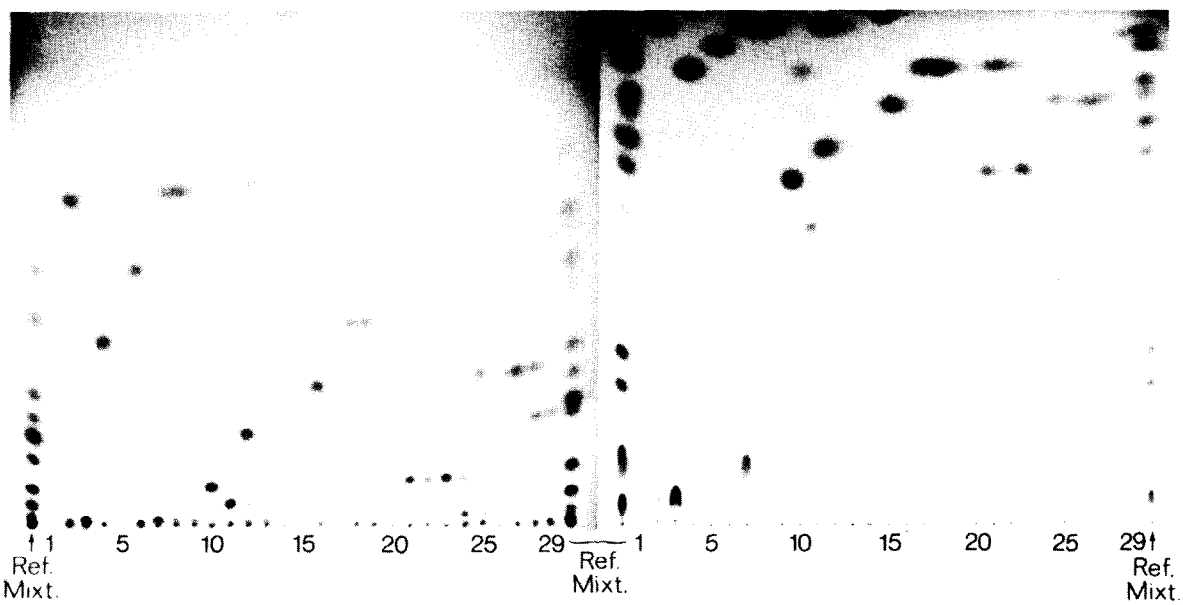

Fig.1. Thin-layer chromatograms [4] on fluorescent silica plates showing the PTH-amino acids corresponding to residues 1–29 of basic pancreatic trypsin inhibitor. Residue one (arginine) and residues 15 and 26 (lysine) remain attached to the resin and were not identified. Residues 5 and 14 (cysteic acid) and residues 17 and 20 (arginine) were also not identified by these procedures. One quarter of the total sample was applied to the plate and developed for approx. 100 min in chloroform (containing 2% ethanol) (A). The same plate was then developed in chloroform–methanol (9:1) (B). The derived sequence (residues not identified in parentheses) is (Arg)–Pro–Asp–Phe–($CySO_3H$)–Leu–Glu–Pro–Pro–Tyr–Thr–Gly–Pro–($CySO_3H$)–(Lys)–Ala–(Arg)–Ile–Ile–(Arg)–Tyr–Phe–Tyr–Asn–Ala–(Lys)–Ala–Gly–Leu, identical to that found previously [11].

of the lysozyme sample. No background was visible after 10 steps of degradation of the insulin B-chain.

The trypsin inhibitor also showed a high coupling yield to the polymer, high coupling efficiency, and low overlap. In this case, the degradation was extended through 29 cycles (fig.1). The complete absence of the PTH-derivative of lysine at positions 15 and 26 indicates that the attachment procedure is essentially quantitative for side-chain amino groups. The PTH-derivatives of histidine, arginine and cysteic acid are not identified by the simple t.l.c. methods used here and also appear as gaps in the sequence. There was no difficulty in identifying the released phenylthiohydantoins and the degradation could easily have proceeded beyond this point.

There appear to be significant advantages in the use of polydimethyl-acrylamide-based supports for solid-phase sequencing. Polypeptides of widely differing molecular weights may be attached to the resin with high efficiency. This may be because the coupling reactions take place in aqueous media in which the peptide or protein samples are soluble and the resin is fully swollen. The degradative efficiency of the sequencing reactions is higher on this support than we have found for amino-polystyrene or amino-propyl-glass. It has been suggested [10] that the limitations of peptide synthesis on polystyrene-based supports may be due, in part, to the dissimilar solvation properties of the polymer matrix and the peptide chain. Thus, under conditions where the resin backbone is extended the polypeptide chain may be folded, and vice versa, resulting in steric hindrance. The fact that a higher repetitive yield is observed on a polar support may indicate that the same considerations apply also to solid-phase peptide sequencing.

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