The Use of Acrylonitrile for the Subtractive Identification of the N-Terminal Residues of Small Peptides

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The elucidation of the primary structures of proteins by chemical methods depends on the determination of the amino acid sequences of peptides produced by more or less specific methods of hydrolysis. This is usually achieved by submitting these peptides to additional partial hydrolytic procedures and separating and characterizing the smaller peptides produced. The separations are generally carried out by paper electrophoresis or paper chromatography, and the peptides may only be available in submicromolar amounts. There are several methods that are sufficiently sensitive and accurate for the determination of the amino acid composition of such small quantities of a peptide, but no entirely satisfactory method is yet available for the determination of its N-terminal residue. The established methods of Sanger (1945) and Edman (1950) are too insensitive to be used at this level. Radiochemical modifications of these methods, and the 'pipsyl' method (Levy, 1957; Corfield, Dilworth, Fletcher & Gibson, 1959), make excessive demands on technique and equipment. The 'DNS' method (Gray & Hartley, 1963) is sensitive enough and extremely valuable, but it is not always reliable, possibly owing to the slowness of the labelling reaction for certain peptides in comparison with the rate of hydrolysis of the reagent. The use of the Edman degradation as a subtractive method on the submicromolar scale has been described by Bahl & Smith (1965), but there exists a need for a simple, sensitive and reliable method for use when stepwise degradation of a peptide is not necessary.

The reaction between acrylonitrile and peptides provides a basis for such a method: addition takes place readily in the presence of bases (Friedman & Wall, 1964), the excess of reagent is easily removed and the N-carboxyethyl derivatives that are produced by hydrolysis of the N-cyanoethylpeptide are stable. The free amino acids remaining in the hydrolysate can be determined by any suitably sensitive method. Since extreme accuracy is not essential, methods involving separation on paper may be used, and a hydrolysate of the unmodified peptide can be run at the same time if required. The only side reactions appear to be the conversion

of non-N-terminal lysine and cysteine into N^ε-carboxyethyl-lysine and S-carboxyethylcysteine (Weil & Seibles, 1961; Friedman, Cavins & Wall, 1965) respectively.

To the dry peptide (0.005–0.025 μ mole) in a soda-glass tube (2 in. $\times$ $\frac{1}{4}$ in.) were added 0.1 M-triethylamine (20 μ l.) and acrylonitrile (5 μ l.). The tube was sealed and the reaction allowed to proceed overnight at 37°. The tube was opened (an ampoule file is convenient) and the contents were evaporated to dryness *in vacuo*. To the residue were added 6 N-HCl (30 μ l.) and, if the peptide contained tyrosine, 0.1 M-phenol (3 μ l.) (Sanger & Thompson, 1963). The tube was re-sealed and heated overnight at 105°. The tube was opened and the contents were evaporated to dryness *in vacuo* over KOH. The amino acids in the hydrolysate of the N-cyanoethylpeptide were determined by the quantitative micro-methods of Blackburn & Lee (1963) (high-voltage paper electrophoresis) or Corfield & Simpson (1965) (high-voltage paper electrophoresis followed by paper chromatography), both of which are based on the measurement of the colour produced when the amino acids on the paper are allowed to react with cadmium-ninhydrin reagent (Heilman, Barollier & Watzke, 1957). The spot corresponding to an amino acid originally present in the N-terminal position but not elsewhere in the peptide was decreased to a negligible intensity, or, for certain amino acids (notably glutamic acid, serine and glycine), to the faint background level that is difficult to eliminate completely when working with peptides eluted from paper strips. When the N-terminal amino acid was present elsewhere in the peptide, the expected decrease in the intensity of the corresponding spot was observed.

The method requires a minimum of manipulation, and is well adapted for dealing with large numbers of samples. It can be applied to a portion of the residual peptide after one cycle of the Edman degradation to identify a second amino acid residue. The method has been tested on peptides containing up to seven amino acid residues. Some were synthetic di- and tri-peptides, and the others were peptides encountered during sequence work on protein fractions isolated from oxidized wool.

No peptides containing *N*-terminal arginine, lysine, cystine or cysteine were included.

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The Hydrolysis of Unimolecular Films of ^{32}P -Labelled Lecithin, Phosphatidylethanolamine and Phosphatidylinositol with Phospholipase A (*Naja naja* Venom)

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Previous studies on the hydrolysis of unimolecular films of lecithin by venom phospholipase A (EC 3.1.1.4) used changes in the surface potential to measure the extent of the breakdown (Hughes, 1935*a*; Colacicco & Rapport, 1965). It was assumed that the lysolecithin formed remained in the film and that the replacement of lecithin by lysolecithin led to a decrease in the surface potential corresponding to the known differences between the surface potentials of films of pure lecithin and lysolecithin (Hughes, 1935*b*).

The present experiments, with a surface radioactivity technique (Bangham & Dawson, 1960), show that when unimolecular films of three ^{32}P -labelled phospholipids are treated with cobra (*Naja naja*) venom a considerable proportion of the lyso-compound formed actually leaves the film. This loss allows the physicochemical and biochemical determinants of the reaction to be investigated more directly.

The apparatus consisted of a cylindrical glass dish (9 cm. diam., 4.5 cm. deep) with a central glass-plate barrier (1 cm. deep) fused across the diameter of the open end and which divided the surface of the aqueous contents into two semi-

circular areas. The vessel was nearly filled with 0.9% NaCl solution (275 ml.) in 0.73 M tris-HCl buffer, pH 7.2, and a solution of the radioactive phospholipid (20 μg . of P/ml.) in CHCl_3 -ether (1:4, v/v) inserted on the surface of one semicircular area with a microsyringe and the solvent allowed to evaporate. The [^{32}P]lecithin and [^{32}P]phosphatidylethanolamine were isolated from ^{32}P -labelled *Saccharomyces cerevisiae* (Dawson & Bangham, 1959; Dawson, 1963). [^{32}P]Phosphatidylinositol was isolated from ^{32}P -labelled *Lipomyces lipofer* by using chromatography on formaldehyde-treated paper (Hörhammer, Wagner & Richter, 1959) for the final purification. The surface radioactivity was measured with a mica-window Geiger-Müller counter coupled to a rate-meter and pen recorder. The surface pressure was recorded with a Wilhelmy (1863) dipping plate and torsion balance. Reagents and venom were inserted into the other side of the vessel and the whole contents were stirred magnetically from the underside.

Fig. 1(*a*) shows a typical result of adding 1 mg. of venom (0.00036%) under a unimolecular film of [^{32}P]lecithin at a pressure of about 30 dynes/cm. ^{32}P was rapidly lost from the film, the surface