A Method for the Quantitative Determination of N-Terminal Amino Acids Based on Radioisotope Dilution

By G. L. CALLEWAERT and C. A. VERNON

Department of Chemistry, University College London, Gower Street, London, W.C. 1

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End-group analysis has often been used together with ultracentrifugation analysis to elucidate protein sub-unit structure. It is also sometimes used to estimate the molecular weight of single polypeptide chains. However, most methods are only semi-quantitative, since handling losses occur in the isolation of derivatives and corrections have to be applied for loss of derivatives during the hydrolysis procedures.

There is now considerable evidence to show that the Edman degradation with phenyl isothiocyanate proceeds quantitatively under certain conditions (Eriksson & Sjöquist, 1960; Edman & Begg, 1967). Consequently, quantitative end-group analysis is possible if errors due to handling losses of the phenylthiohydantoin derivatives can be avoided. A procedure that gives good results has been described by Eriksson & Sjöquist (1960), but it involves exacting experimental techniques. We now report the details of a relatively simple method in which handling losses are avoided by the use of radioisotope dilution.

Two samples of salt-free protein (approx. 0.25 μ mole) are weighed out on a microanalytical balance (e.g. Cahn Gram Electro Balance). One sample is heated to constant weight at 110° to determine moisture content. The other is dissolved in 1 ml. of propan-1-ol-water (3:4, v/v) containing *N*-dimethylallylamine (0.4 M) and enough trifluoroacetic acid to give pH 9.5 (apparent). This solvent system is similar to that described by Edman & Begg (1967). Phenyl [³⁵S]isothiocyanate (20 μ l., specific activity approx. 0.5 mc/m-mole; The Radiochemical Centre, Amersham, Bucks.) is added and coupling to the protein achieved by maintaining the mixture at 40° for 1.5 hr. Excess of phenyl isothiocyanate is then extracted by repeated washing with benzene and the remaining liquid removed by freeze-drying or rotary evaporation. The phenylthiohydantoin derivative is now formed under the conditions described by Eriksson & Sjöquist (1960), i.e. water (1 ml.) and acetic acid (1 ml.) saturated with HCl are added and the mixture is maintained, with stirring, at 40° for 2 hr.

Isotope dilution is now carried out by adding a known amount of the appropriate unlabelled

phenylthiohydantoin (approx. 2.5 μ M) dissolved in ethanol (1 ml.). It remains only to isolate a pure sample of the phenylthiohydantoin and to determine its specific activity. This is best done by chromatographic procedures, since, as Cherbuliez, Baehler, Lebeau, Sussmann & Rabinowitz (1960) have shown, not all the radioactivity is contained in the desired derivative.

The mixture, after being evaporated nearly to dryness on a rotary evaporator, is suspended in water (2 ml.) and extracted twice with ethyl acetate (2 ml.). The resulting extract is chromatographed as a band on ethanol-washed silica-gel-impregnated paper (Whatman SG 81) with chloroform, chloroform-methanol (9:1, v/v) or chloroform-formic acid (20:1, v/v) as solvent, the choice depending on the particular phenylthiohydantoin (Brenner, Niederwiesser & Pataki, 1961). The appropriate band is eluted and rechromatographed with a second solvent system. The band finally obtained is eluted with a convenient amount of ethanol (approx. 10 ml.) and the concentration of phenylthiohydantoin in the resulting solution is determined spectrophotometrically (269 m μ) by using data given by Sjöquist (1957). A portion (3 ml.) of the solution is added to toluene (15 ml.) containing 0.5% (w/v) of the scintillator 2-(4-*tert*-butylphenyl)-5-(4-biphenyl)-1-oxa-3,4-diazole [Ciba (A.R.L.) Ltd., Duxford, Cambs.] and radioactivity is then counted with a calibrated liquid-scintillation counter. The equivalent weight, *M*, of the protein can now be calculated from the equation:

$$M = w(S_1 - S_2)/S_2N$$

where *w* is the dry weight (g.) of protein taken, *S*₁ and *S*₂ are the specific activities of the phenyl [³⁵S]isothiocyanate and of the isolated phenylthiohydantoin respectively, and *N* is the number of moles of added unlabelled phenylthiohydantoin.

The results obtained with bovine serum albumin are illustrative. Independent determinations gave the following values for *M*: 69 200, 71 000, 70 500, 69 500, 70 000 and 68 900. The mean value, 69 850, has a standard deviation of 800 and agrees very well with the value (69 000) obtained by ultracentrifugation (Gehatia, 1960). Bovine serum albumin

provides a good test for the method, since (a) it has *N*-terminal aspartic acid (Desnuelle, Röver & Fabre, 1951), which is relatively difficult to remove quantitatively, and (b) it has a large equivalent weight. The method is, of course, applicable only when the protein consists of a single chain or of an aggregate of identical chains.

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