A Method for Determination of the Amino Acid Sequence of Proteins

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Edman¹ has proposed an ingenious method for stepwise degradation of peptides by means of phenyl isothiocyanate. It has now been attempted to adapt this method for use with proteins.

The reaction occurs in two steps, as indicated:

Step I occurs under similar conditions to those of other protein amino end group methods^{2,3}, *i.e.* in aqueous pyridine at pH 9 and 40°. Step II may be carried out in aqueous hydrochloric acid (0.6–1.2 *N*), a modification which was suggested by M. Ottesen for the analysis of peptides. In contrast to other methods, hydrolysis of the protein is not required for identification of the terminal amino acid. Actually, the number of end groups seemed not to be increased upon subjecting the protein to the reaction for a second and third time, which indicates that measurable hydrolysis of peptide bonds had been avoided. There appears therefore to be no necessary limit to the complete elucidation of the peptide sequence of a protein.

The proteins used for this preliminary study of the method were insulin, ovomucoid, conalbumin, and β -lactoglobulin.

Insulin has yielded 3–3.5 end groups per subunit of MW 12 000. Recovery experiments in which tripeptides were analysed by the same technique suggest that losses of 15–20 % must at present be counted on; thus the value for the end groups of insulin appears to approach that found by the DNP method (4 per 12 000)². Similarly in β -lactoglobulin we have found about 2.5 (uncorrected) while Porter found 3 terminal amino groups (corrected value) by the DNP method⁴. With the two other proteins, approximately one end group was found, in agreement with DNP-results⁵.

Qualitatively, phenylalanine and glycine,

but, unexpectedly, also small amounts of alanine were found in insulin, alanine in ovomucoid, and leucine and some glycine in β -lactoglobulin.

Identification of the second and third amino acids from the amino end is as yet incomplete, but the results appear to be compatible with those of Sanger in the case of insulin⁶.

The technique is briefly as follows: Step I is performed exactly according to Edman at a constant pH of 9.0 or 9.8 for 0.5–3 hours; also the extraction with benzene is the same. The product is then suspended or dissolved in little water and continuously extracted with ether until no more U.V. absorbing material is eluted. Addition of guanidine halide (1.4 g/ml of the aqueous solution) is of advantage during this extraction. It seems to be necessary for the subsequent quantitative extraction of the thiohydantoin, which

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is formed upon the addition of 6 N HCl to final concentrations of 0.6–1.2 N (Step II). The thiohydantoin which appear in the ether after acidification of the reaction mixture, show a characteristic maximum at 267–268 μ , with molar extinction coefficient close to 16 000. Determination of the number of terminal amino acids is based on this property. Identification is done chromatographically after regenerating the amino acid by baryta hydrolysis according to Edman^{1,2}.

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On the Use of Rayleigh-Calvet-Philpot Interference Fringes for the Measurement of Diffusion Coefficients According to the Moment Method

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The astigmatic modification of the Rayleigh interferometer, characterized by giving interference fringes which take the form of the refractive index function in the cell, was described by Philpot and Cook¹ in 1948. The author has therefore previously called these fringes Rayleigh-Philpot-Cook fringes. However, the optical arrangement described by Calvet²⁻⁴

earlier contains the essential features of this interferometer. This fact has hitherto escaped the author's attention, largely because it was concealed by Calvet's addition of some nonessential features and of a slit and rotating drum arrangement. Hereby he produced interferograms with the cell coordinate and time on two perpendicular axes, which have quite another appearance than Philpot-Cook's interferograms with the cell coordinate and the refractive index on these axes. Although Calvet's priority is undisputable, Philpot and Cook have no doubt carried out their work independently. It is therefore suggested that the fringes in question be called as in the heading of this article. These fringes are a special kind of *integral interference fringes*, which are produced by bringing to interference one light beam passing through the cell and another passing through a comparison cell with a constant refractive index, whether the basic interferometer is according to Rayleigh or of some other kind. Hence the observation methods of Labhart and Staub⁵ and Antweiler⁶ also belong to the integral fringe methods. On the other hand, the Gouy method (Kegeles and Gosting⁷; Longworth⁸; Coulson, Cox, Ogston and Philpot⁹) and the gradient-recording method described recently by the author¹⁰ might be called *differential interference methods*, and the fringes *differential interference fringes*, since the two interfering light pencils are both passing through the cell.

In a previous article¹¹, the author described the calculation of diffusion coefficients from integral fringe interferograms according to two methods, the conventional height-area method (this name, of course, refers to the gradient curve), and a method based upon a direct comparison between the position of the fringes and the course of the integral of the error function. In this article, the calculation of the diffusion coefficient according to the moment method will be outlined.