IDENTIFICATION OF THE BLOCKED N-TERMINUS OF AN ALCOHOL DEHYDROGENASE FROM *DROSOPHILA MELANOGASTER* N-11

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1. Introduction

The use of mass spectrometry in peptide sequence determination has become more popular in recent years. Although requiring more material than wet chemical methods (e.g., dansyl-Edman degradations) mass spectrometric sequence strategies compensate for this by analysing mixtures of peptides and thereby avoiding losses due to extensive purification of peptides. It also differs from classical techniques in not requiring a free amino terminus. When the presence of a blocked N-terminus is suspected in a peptide or protein, acid hydrolysis followed by gas–liquid chromatography, identification of the fatty-acid liberated may be employed [1]. Alternatively, hydrazinolysis may be used, and the N-terminal group identified as the corresponding hydrazide [2]. Frequently, it is only after one, or both, of these methods have failed, or given ambiguous results, that a solution by mass spectrometry may be sought. In such cases, only small amounts of peptide may be available.

This was the situation with the peptide described here and only 50 nm were available. Typically, 100–200 nm are required [3], although subsequently [4] 70–200 nm have been used for mass spectrometric identification of blocking groups.

2. Materials and methods

The enzyme was isolated from an isogenic strain of *Drosophila melanogaster* N-11 (supplied by Dr R. G. Camfield, University of Nottingham) by the method in [5]. The purified, oxidized enzyme was digested with trypsin and the peptides fractionated by gel filtration on Sephadex G-25 SF in 6% (v/v) acetic acid. A low molecular weight peptide was isolated by paper chromatography and electrophoresis, at pH 3.5, as in [6]. This peptide, although capable of reaction with ninhydrin, gave only the *e*-dansyl lysine derivative on treatment with dansyl chloride in aqueous acetone, and failed to degrade by the dansyl-Edman procedure [7]. The peptide was then subdigested with chymotrypsin and the peptides generated isolated by paper electrophoresis, at pH 6.5. An acidic, ninhydrin negative peptide, having the amino acid composition Ser, 1.0, Phe, 1.0, was located by the starch iodine method [8].

The peptide (50 nm) was acetylated and then permethylated prior to mass spectrometric analysis. Acetylation was carried out according to [9] using 0.5 ml of the reagent [U-^{2}H]acetic anhydride in methanol (1:4, v/v) for 3 h at room temperature. The short permethylation conditions of [10] were adopted; the peptide was dissolved in 20 μl dimethyl

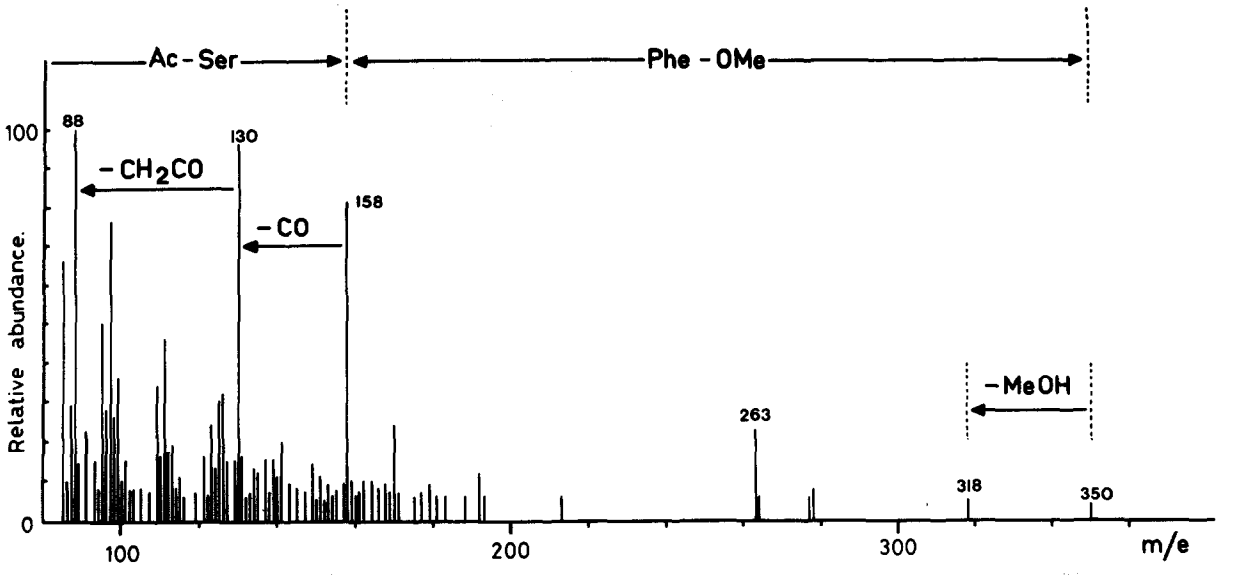

Fig.1. Partial mass spectrum of the acetylated and permethylated peptide at a source temperature of 145°C.

sulphoxide and reacted with 40 μl methyl sulphinyl carbanion base (an excess judged by the colouration of triphenylmethane paper [11]), followed by 40 μl methyl iodide. Low resolution mass spectra were recorded on an AEI MS902 instrument operating at an accelerator voltage of 8 kV and at an electron beam energy of 70 eV.

3. Results and discussion

Five spectra were recorded in the source temperature range 120–170°C. The spectrum taken at a temperature of 145°C is shown on fig.1. The peak at *m/e* 158 indicates an N-terminal serine residue which does not carry an isotopically labelled acetyl group. A naturally occurring acetyl blocking group is therefore indicated. The dipeptide is identified as acetyl Ser–Phe by the peak at *m/e* 350.

We have shown that 50 nm peptide is sufficient to identify a blocked N-terminal group. The decrease of signal intensity with increasing *m/e* ratio, however, indicates that more material would normally be required for further sequence identification of larger peptides. Yet, if mass spectrometry were used in conjunction with classical peptide sequencing studies, an identification of the N-terminus would suffice. Although 50 nm may be regarded as a relatively large amount of peptide for the determination of a short sequence, current use of other methods for the identification of N-terminal blocking groups requires similar quantities [12].

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