The Identification of a Mutant Peptide of an Abnormal Haemoglobin by Mass Spectrometry

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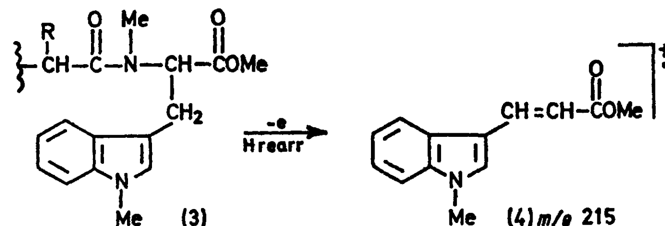
Summary A mutant peptide from an abnormal haemoglobin, contaminated by a second peptide, has been sequenced by mass spectrometry; the spectrum of one derivatised mixture allowed the sequences Leu-Leu-Gly-Asn-Val-Leu-Phe and Leu-Leu-Val-Val-Tyr-Pro-Trp to be determined.

MASS SPECTROMETRY has been widely used as a method for the sequence determination of peptides.[1] We now report the identification of a mutant peptide of an abnormal haemoglobin by mass spectrometry.

The globin from the abnormal haemoglobin (Hb Peterborough) was precipitated using acidic acetone, aminoethylated, and digested with trypsin.[2] Fingerprinting indicated the presence of an abnormal peptide, amino-acid analysis of which established the abnormality to lie in the replacement of a valine by phenylalanine in the "normal" sequence Leu-Leu-Gly-Asn-Val-Leu-Val-Cys.[2] Since the procedure for derivative formation which we wished to employ for mass spectrometry does not give volatile derivatives for peptides containing aminoethylcysteine residues, the mutant peptide was separated from all other tryptic peptides, excepting one, by ascending chromatography and treated with chymotrypsin for 45 min at 37°



and pH 8·6.[2] The mixture of peptides obtained was then N-acetylated with acetic anhydride–methanol[3,4] and per-

methylated by successive treatment with dimethyl sulphoxide anion in dimethyl sulphoxide and methyl iodide.[4,5] Since under the conditions of the chymotryptic digest, chymotrypsin hydrolyses on the *C*-terminal side of phenylalanine, partially after leucine, but not after valine, the volatile peptides which may be produced after derivatisation of the two possible mutant peptides are as indicated in the Table.

250° can be extended (Figure 2). The only mass difference from *m/e* 764 to another abundant ion, which corresponds to the derivative of an amino-acid, is 161 (to *m/e* 925), indicating phenylalanine. Thus the sequence Leu-Leu-Gly-Asn-Val-Leu-Phe is established, where the Phe is *C*-terminal (M^+ at *m/e* 956, M^+—CH_3 at *m/e* 941, M^+—OCH_3 at *m/e* 925). This confirms the mutation Val → Phe at residue number 111 rather than at 109 (Table).

TABLE

Possible volatile peptides from the derivatisation of chymotryptic peptides originating from the mutant[a]

Sequence No	105	106	107	108	109	110	111	112
Possible mutant (1)	Leu	Leu	Gly	Asn	Phe	Leu	Val	Cys
Volatile peptides[b]	Leu	Leu	Gly	Asn	Phe			
	Leu	Leu	Gly	Asn	Phe	Leu		
Possible mutant (2)	Leu	Leu	Gly	Asn	Val	Leu	Phe	Cys
Volatile peptides[b]	Leu	Leu	Gly	Asn	Val	Leu		
	Leu	Leu	Gly	Asn	Val	Leu	Phe	

[a] Arrows in the Table indicate sites of hydrolysis (→) or partial hydrolysis (→) by chymotrypsin; [b] For brevity, the normal amino-acid abbreviations are used to indicate the *N*-acetylated permethylated derivatives.

The partial mass spectrum (*m/e* 140—770, excepting the regions *m/e* 320—350, 425—490, 645—690, where no abundant ions occur) of the derivatives of the mixture as obtained at a source temperature of 250° (Figure 1) establishes two independent sequences Leu-Leu-Gly-Asn-Val-Leu and Leu-Leu-Val-Val-Tyr. Moreover, fractionation of the peptide mixture can be achieved on the probe,[4] and in spectra obtained at a source temperature of 200°, the sequence ions due to the peptide Leu-Leu-Gly-Asn-Val-Leu are of much greater abundance (relative to those of the other sequence), and there is evidence [M^+ at *m/e* 795 (764 + OCH_3)] that one of the peptides volatilising at 200° is Leu-Leu-Gly-Asn-Val-Leu containing *C*-terminal leucine [*i.e.* indicates the mutant (2) Table].

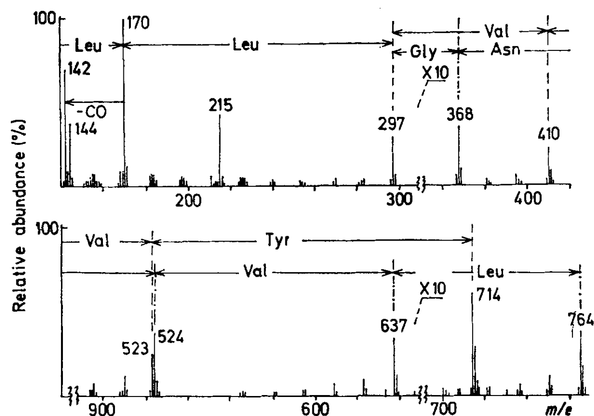


FIGURE 1. *Partial mass spectrum of the derivatised peptide mixture, obtained at a source temperature of* 250° *and* 70 eV.

By running the high mass region (*m/e* 700—1060) of the spectrum obtained at 250° at high multiplier gain, the sequence of both peptide derivatives which are volatile at

The sequence of the contaminating peptide Leu-Leu-Val-Val-Tyr can also be extended (Figure 2). No sequence ion

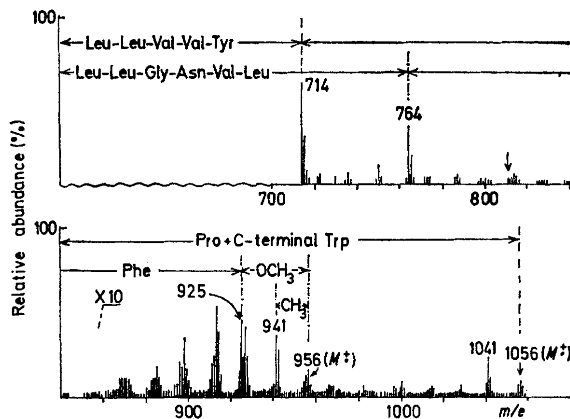


FIGURE 2. *High mass region* (*m/e* 700—1060) *of the derivatised peptide mixture* (*conditions as in Figure* 1).

following the tyrosine residue can be identified, but it can be deduced that this peptide contains *C*-terminal tryptophan from the abundant ions (Figure 1) at *m/e* 144 and 215 [see (3) and (4)].

The last abundant ion in the scan at 250° occurs at *m/e* 1056 (Figure 2), and the inference that this is a molecular ion is supported by an abundant *m/e* 1041 peak (M^+—CH_3). Since the derivative of tryptophan has a *C*-terminal mass of 245, the 'missing' amino-acid after derivative formation has a mass of 1056 − (714 + 245), *i.e.* 97, which corresponds to a proline residue. The full sequence of the contaminating peptide from the tryptic digest is therefore Leu-Leu-Val-Val-Tyr-Pro-Trp. It is noteworthy that under the conditions used chymotrypsin would not be anticipated to cleave at the *C*-terminal side of tyrosine

when proline is the next residue. In our experience, it is common for the sequence ions following proline residues to be of low (and occasionally negligible) abundance.

The sequences deduced by mass spectrometry have been independently deduced by classical means.[2]

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