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Haemoglobin Södertälje (β 118 Phe→Val): A New Mutation in Human Haemoglobin Identified by Electrospray Ionisation Mass Spectrometry

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Electrospray ionisation mass spectrometry (ESI-MS) is a rapid analytical tool for identification of hundreds of haemoglobin (Hb) variants¹⁻⁴. This far, the ESI-MS usage has led to the discovery of over 60 new mutations in the human Hb⁵. The study of Hb abnormalities has enriched understanding of the structure-function relationship of the Hb molecule. In this short communication, we describe a new mutation in the β -chain, named Hb Södertälje, that is clinically silent.

The sample was encountered during routine haematological investigations, i.e. full blood count, isoelectric focusing and HPLC analyses. For mass spectrometric analysis of the intact globins, whole blood sample collected in ethylene diamine tetraacetic acid tube and then diluting to a 500 fold with 50% aqueous acetonitrile (0.2% formic acid) was prepared⁶. The globin chains were reduced with dithiothreitol and carboxymethylated using iodoacetamide prior to digestion with trypsin as previously described⁷. A tandem quadrupole (Quattro micro^{TM}) and a quadrupole time-of-flight mass spectrometer (Waters MS Technologies, Manchester, UK) equipped with nano electrospray ionisation source were used.

DNA extraction and amplification of 1612 bp covering all three exons were performed as described previously⁸. The sequencing was performed using 5'-TTCTGCTTTTATTTTATGGTTGG-3' using the Big Dye Terminator DNA sequencing kit (Applied Biosystems, CA, USA).

A routine haematological investigation indicated presence of a variant Hb slightly anodal to Hb A. The mass spectrum (transformed to a mass scale) of the intact globins is shown in figure 1. This spectrum revealed a β -chain variant (β^x , 15819.4 Da) ~48 Da lighter than the mass of the β -chain (15867.2 Da). Based on the relative intensities alone the variant constituted ~37% of the total β -chains, i.e. $\beta^x/(\beta^x+\beta)$.

There are two possible amino acid substitutions for a –48 Da mass change, namely Phe→Val and Tyr→Asp. There are eight Phe and three Tyr residues in a β -chain. Tandem mass spectrometry (MS/MS) on the intact β^x -chain performed as described previously⁹ directed the amino acid substitution to residues 111-124 (VCVLAHHFGKEFTP). These residues fall within tryptic β T12 and β T13 peptides. The mass spectrum of the trypsin digest

of carboxymethylated (CAM) globins demonstrated a variant form of β T12 peptide. The doubly protonated variant β T12 ($\beta^{\text{xCAM}}\text{T12}^{2+}$) peptide (m/z 864.94) was 24 m/z units lower than the normal $\beta^{\text{CAM}}\text{T12}^{2+}$ peptide mass (m/z 888.94) (Fig. 2). In a normal β T12 peptide (LLGNVLVCVLAHHFGK), there is only one Phe residue and no Tyr residue. MS/MS experiments on the $\beta^{\text{xCAM}}\text{T12}^{2+}$ peptide ions showed y ions ($y_5 - y_{12}$) shifted by $-48 m/z$ from the corresponding y-ions of the $\beta^{\text{CAM}}\text{T12}^{2+}$ peptide ions (Fig. 3). The MS/MS data thus indicated that the amino acid exchange occurred in the last five residues of the β T12 peptide. A close analysis of the MS/MS data localized the modification resulting in the variant by the y_3 ions being shifted by $-48 m/z$ units (data not shown). This confirmed the amino acid substitution on the third residue from the C-terminus of the β T12 peptide corresponding to β 118 Phe $\rightarrow$ Val substitution in the β -chain. DNA sequencing unequivocally confirmed the T $\rightarrow$ G (i.e. TTT $\rightarrow$ GTT) mutation. This is a new amino acid substitution at this position, named Hb Södertälje as the patient is a resident of Södertälje, a southern suburb of Stockholm, Sweden. There are two other Hb variants, Hb Harrow (β 118 Phe $\rightarrow$ Cys)10 and Hb Minneapolis (β 118 Phe $\rightarrow$ Tyr)11, known to occur at the same position. Both of these Hb variants, like Hb S Södertälje, do not alter the normal function of the Hb molecule.

ESI-MS is a rapid technique to identify Hb variants requiring minimal sample work-up. The technique is able to demonstrate electrophoretically 'silent' Hb variant. This has an advantage for analysis of amino acid substitution resulting in no net charge difference that could be overlooked by electrophoresis and HPLC techniques.

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Figure 1. Mass spectra transformed to a true mass scales of (a) normal Hb and (b) Hb sample carrying a β -chain variant (β^x). The β^x is ~48 Da lower in mass than the β -chain.

Figure 2.

Part of the mass spectra of tryptic peptides from carboxymethylated (CAM) globin chains from (a) normal Hb and (b) Hb variant showing doubly protonated variant $\beta T12$ ($\beta^{x^CAM}T12^{2+}$) peptide 24 m/z units lower than the corresponding normal $\beta^{CAM}T12$ peptide.

Figure 3.

Tandem mass spectra from the (a) normal $\beta^{\text{CAM}}\text{T12}^{2+}$ peptide and (b) $\beta^{\text{xCAM}}\text{T12}^{2+}$ peptide ions. The -48 m/z units change in the y_5 - y_{12} ions in (b) with respect to (a) directs the amino acid substitution within the five C-terminal residues of the βT12 peptide.