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Note

Identification of the α chain abnormal hemoglobin Jackson (α127 Lys→Asn) after isolation of the core peptide by high-performance liquid chromatography

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Identification of hemoglobin (Hb) variants with a substitution in the "core segment" of the α polypeptide chain (residues 100–139, inclusive) is often complicated by difficulties in isolating the tryptic peptide αT-12 (residues 100–127, inclusive) and to a lesser extent peptide αT-13 (residues 128–139, inclusive) in pure form. A common approach is to remove these peptides at pH 6.5 from a tryptic digest by centrifugation, oxidize this precipitate with performic acid, digest the oxidized core with chymotrypsin, and separate the resulting fragments by electrophoresis combined with chromatography, or by chromatography including high-performance liquid chromatography (HPLC)[1]. In this communication we report the identification of Hb Jackson, an α chain variant with a Lys→Asn substitution at position α127, which was possible because the core peptide αT-12,13 was readily isolated from a tryptic digest of the oxidized α chain by HPLC.

MATERIALS AND METHODS

The families

Three members of a black family from Savannah, GA, U.S.A., and one member of a second family from Albany, GA, U.S.A., had the "fast moving" Hb J type of variant which was readily observed by starch gel or cellulose acetate electrophoresis at alkaline pH but not by citrate agar electrophoresis at pH 6.2 (for methodology, see ref. 2). The four Hb J heterozygotes were perfectly healthy black adults; their hematological data were completely normal (Table I). The quantity of the variant in the red cells of each subject was determined by DEAE-cellulose chromatography[3], and averaged about 20% (the chromatographic method allowed only a partial separation of the Hb J and the normally occurring minor Hb A_1 components). About 0.5% of Hb J_2, *i.e.* a variant of Hb A_2 containing an abnormal α chain ($\alpha_2^J\delta_2$), was also present.

* Contribution Number: 0754.

Methodology

DEAE-Cellulose chromatography[3] was used to isolate a larger quantity of Hb J from a red cell lysate. The isolated Hb was contaminated with a small amount (about 10%) of Hb A_1. The α-J and β chains were separated by CM-cellulose chromatography[4], and some of the α-J chain was oxidized with performic acid according to the method of Hirs[5]. Both the untreated α-J chain and the oxidized α-J chain were digested with TCPK-trypsin (Worthington, Freehold, NJ, U.S.A.) for 4 h, at pH 8.8, and at room temperature. The pH of the digests was adjusted to 2.5 with 1 *M* hydrochloric acid and the tryptic peptides were separated by HPLC using methodology described before[1].

The solvents and the program used were not altered except for extending the T-P period (*i.e.* elution with 100% of solvent A which contains 50% acetonitrile in 0.01 *M* ammonium acetate, pH 5.7) at the end of the chromatographic experiment from 20 to 50 min. Each isolated peptide was analyzed with a Beckman 121M amino acid analyzer equipped with a system AA computing integrator.

The isolated T-12,13(ox) peptide was digested with chymotrypsin, and the resulting fragments were separated by HPLC (for details, see ref. 1). Amino acid analyses and sequence analyses using the ultramicrosequencing procedure described by Chang *et al.*[6] readily identified the peptides.

RESULTS AND DISCUSSION

The HPLC chromatogram depicting the separation of the soluble peptides from a digest of the untreated α-J chain was identical to that published before (Fig. 1 or ref. 1). The location and the amino acid composition of each of the following peptides T-1, T-1,2, T-2, T-3, T-4, T-5, T-6, T-7, T-8, T-9, T-8,9, T-10, T-11 and T-14 were not different from those observed for corresponding fragments of the normal α-A chain. Peptides T-12 and T-13 were not uncovered although a minute quantity of the T-13 peptide was observed in a few chromatograms where it eluted some 8 to 10 min ahead of peptide T-6.

Fig. 1 illustrates a similar chromatogram but of the peptides from a tryptic digest of the oxidized α chain. The effect of oxidation was considerable. Peptide T-3 which contains the only tryptophan residue of the α chain was destroyed. Partial oxidation of the methionyl residues in peptides T-5 and T-9 made it nearly impossible to separate these fragments from each other and from peptide T-6. However, oxi-

TABLE I

HEMATOLOGICAL AND HEMOGLOBIN COMPOSITION DATA FOR FOUR Hb JACKSON HETEROZYGOTES

Subject	*Age*	*Hb (g/dl)*	*PCV (l/l)*	*RBC (10^{12}/l)*	*MCV (fl)*	*MCH (pg)*	*MCHC (%)*	*Hb A_2* (%)*	*Hb X_2* (%)*	*Hb X* (%)*
J.R.	34	15.1	0.428	4.24	101	36.3	34.8	2.9	0.6	21.8
T.R.	14	15.4	0.453	4.93	92	31.6	33.3	2.5	0.5	19.6
G.R.	10	12.8	0.396	4.68	85	27.7	31.6	2.3	0.5	21.2
M.P.	16	15.3	0.463	5.50	84	28.2	32.5	2.0	0.5	16.0

* By DEAE-cellulose chromatography[3].

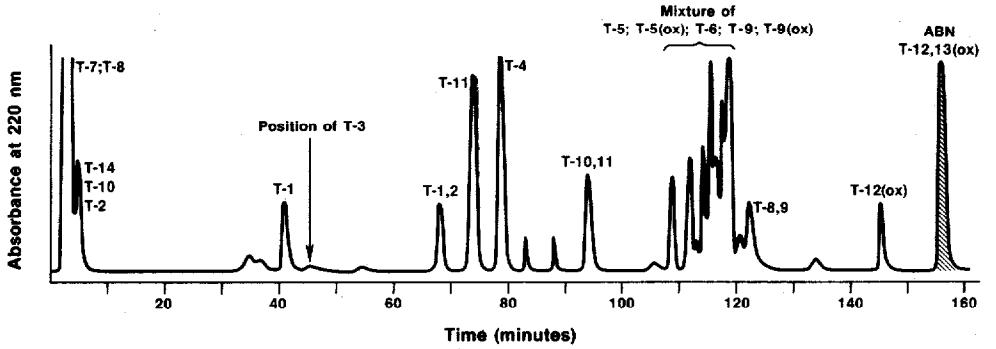

Fig. 1. Separation by HPLC of peptides from a tryptic digest of the α chain of Hb Jackson oxidized with performic acid prior to digestion. See text for further details.

dation of the cysteinyl residue of peptide T-12 to cysteic acid (position 104 of the α chain) improved the solubility of this core peptide which is readily eluted as a single peak in the later part of the chromatogram. Two such zones were observed (Fig. 1). One small peak was eluted at about 145–146 min and was identified as normal T-12 (this peptide likely originated from the small amount of contaminating normal α-A chain). The last zone eluted between 155 and 158 min, was a combination of peptides T-12 and T-13, and had the following composition: Cysteic acid: 0.81 (1), Asp: *2.37* (1), Thr: 3.78 (4), Ser: 4.79 (5), Glu: 1.29 (1), Pro: 2.22 (2), Ala: 5.79 (6), Val: 3.52 (4), Leu: 8.70 (9), Phe: 2.00 (2), His: 3.17 (3), Lys: *1.12* (2). These data clearly indicate a Lys→Asx substitution in this 40 amino acid residues containing peptide.

These results together with the electrophoretic and chromatographic data of the intact abnormal Hb identify the substitution as being a Lys→Asn (as present in Hb Jackson) rather than a Lys→Asp replacement. It also is worth noting that a Lys→Asp replacement would require two base substitutions in the DNA while only one is needed for the Lys→Asn replacement. The loss of a lysyl residue at position α-127 eliminates the digestion site at that position, resulting in the formation of the combined T-12,13 fragment. This conclusion was further supported by the results of analyses of the chymotryptic fragments of the T-12,13(ox) core peptide. Fig. 2 illustrates the separation of these peptides by HPLC, and identifies the location of each fragment in the intact α chain through their position numbers. One fragment (identified by position numbers 123–127, inclusive) had the following composition: Asp *1.86*(1), Ser 1.21(1), Ala 1.16(1), Val 0.71(1), Leu 1.00(1), Lys *0*(1) while sequence analyses (Ala-Ser-Leu-Asp-Asn) identified the Lys→Asn substitution at the fifth position of this peptide or at position 127 of the intact α chain.

Hemoglobin Jackson was discovered by Moo-Penn *et al.*[7] who also observed this abnormality in a black female. Our data support observations reported in that study indicating that the α127 Lys→Asn substitution does not lead to altered functional properties nor causes clinical abnormalities. The same appears to be the case for the Hb St. Claude, an α chain abnormality with a Lys→Thr substitution at position α127[8].

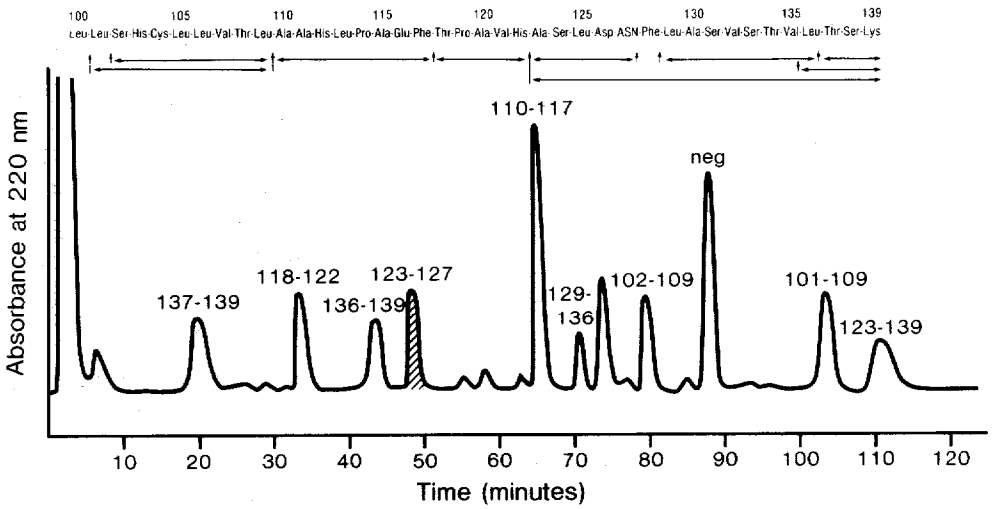

Fig. 2. Separation by HPLC of peptides from a chymotryptic digest of the αT-12,13(ox) core peptide of Hb Jackson. The numbers identify the positions of the fragments in the intact α chain. The 123–127 peptide was sequenced and contained an extra Asn residue while a Lys residue was absent.

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