HEMOGLOBIN DEACONESS
A NEW DELETION MUTANT: β131 (H9) GLUTAMINE DELETED

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SUMMARY: Hemoglobin Deaconess was detected as a band migrating in the position of fetal hemoglobin when an hemolyzate was electrophoresed on cellulose acetate at pH 8.4. This abnormal hemoglobin also migrates between Hb S and C on citrate agar electrophoresis at pH 6.2. Chemical characterization of this mutant hemoglobin shows glutamine is deleted at position 131 in the β-chain. Initial data indicates that the stripped hemoglobin has a reduced oxygen affinity with a Hill constant of n=2.0.

INTRODUCTION

Studies of the structure-function relationship of abnormal hemoglobins have provided invaluable information about areas of the molecule that are critical to its function. Of the more than 230 abnormal hemoglobins described, most are single amino acid substitutions in the polypeptide chains. A deletion mutation is a rarer event and involves the deletion of one or more nucleotide triplet codes (1). The chemical characterization of a new deletion mutant detected in a 49-year old black female is described in this report.

MATERIALS AND METHODS

Electrophoretic and other routine methods used in the detection and diagnosis of abnormal hemoglobins have been described (2). The abnormal hemoglobin was separated on DEAE-Sephadex by the method of Huisman and Dozy (3). Chain separation and peptide mapping were carried out according to the procedure of Clegg et al (4). The isolated mutant β-chain was aminoethylated (5) and digested with TPCK°-trypsin; the peptides were then separated by column chromatography (6).

Amino acids were analyzed on a Beckman 121 Amino Acid Analyzer (7), after the peptides were hydrolyzed in 6 N HCl at 110°C for 24

° TPCK L-(1-Tosylamido-2-phenyl) ethyl chloromethyl ketone

hours. Tryptic peptide XIII after reaction with 4-sulfophenylisothiocyanate (9) was subjected to automated Edman degradation (8) on a Beckman Model 890 C Sequencer; the DMAA* fast peptide program provided by the manufacturer was used (10). The PTH+-amino acids were identified on a Beckman Model 65 gas chromatograph (11) and confirmed by thin layer chromatography (12) and hydrolysis with HI (13). Carboxypeptidase A and B, treated with DFP, were used to determine the carboxyl terminal sequence (14).

All enzymes were obtained from Worthington Biochemical Corp., and all other reagents used were of the highest purity.

RESULTS AND DISCUSSION

Figure 1 shows the results obtained when a hemolyzate from the patient was subjected to electrophoresis at pH 8.4 on cellulose acetate and at pH 6.2 on citrate agar. At pH 8.4 the abnormal hemoglobin migrates in the position of fetal hemoglobin (Hb F), whereas, at pH 6.2 it appears as a band between Hb S and C. The concentration of Hb F is 2% as determined by the Singer method.

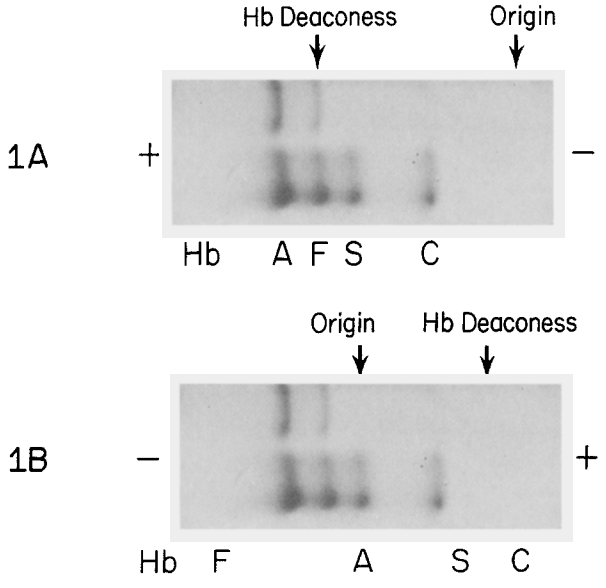

Fig. 1 Electrophoretic patterns at pH 8.4 (1A) on cellulose acetate membrane and at pH 6.2 (1B) on citrate agar.

* DMAA Dimethylallylamine (N-allyl-N, N-dimethylamine)
+ PTH Phenylthiohydantoin

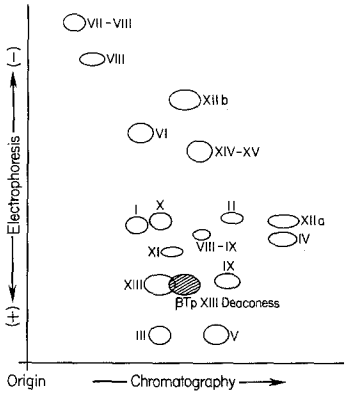

Fig. 2 Peptide map of a tryptic digest of aminoethyl-β chain from Hb Deaconess. Chromatography was in pyridine, butanol-1, acetic acid, and water (50:75:15:60). Electrophoresis was at pH 4.7 for 2 1/2 hours at 34 V/cm. The hatched area indicates the position of abnormal TpXIII.

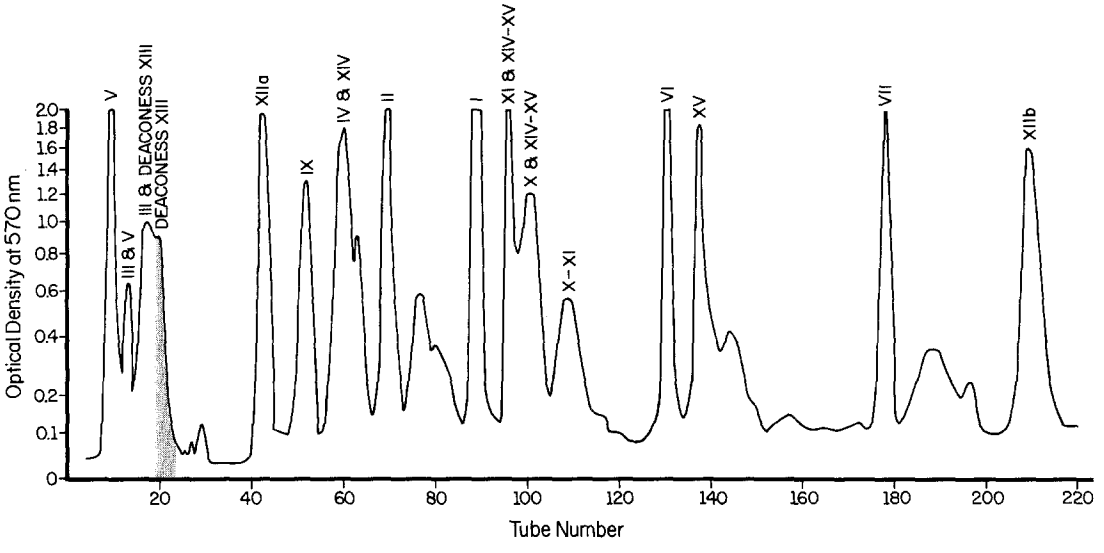

Fig. 3 Elution of tryptic peptides from a column (0.9 x 23 cm) of BioRad A5 resin. A linear pyridine acetate gradient (0.2M, pH 3.1, to 2M, pH 5.0) was used to elute the peptides at a flow rate of 30 ml per hour. Ten percent of the effluent was continuously monitored at 570 nm after reaction with ninhydrin.

Quantitation of the hemoglobins from a preparative DEAE-Sephadex column results in 3.3% Hb A_2, 29.0% Hb Deaconess, and 67.7% Hb A.

Separation of the mutant chain by the Clegg procedure indicates that it is a slow-moving β-chain. The peptide map shows a normal distribution of peptides except that βTpXIII migrates faster in the chromatography dimension (Fig. 2).

Figure 3 shows the separation of a tryptic digest of the abnormal β-chain. Tryptic peptide XIII elutes slower from the column and was isolated pure. In contrast, normal βTpXIII is a faster migrating peptide and coelutes with βTpIII. The amino acid composition of Hb Deaconess βTpXIII is normal except for the loss of a single residue of glutamic acid (Table I). Similar results were also obtained by elution and hydrolysis of abnormal βTpXIII from a peptide map.

The sequence of βTpXIII has one residue of glutamic acid at

TABLE I

Amino Acid Composition of βTpXIII

Amino Acid	Normal βTpXIII	Hb Deaconess βTpXIII
Lys	1	0.9
Thr	1	1.0
Glu°	3	2.1
Pro	2	1.8
Ala	2	2.0
Val	1	1.2
Tyr	1	0.8
Phe	1	1.0

° Amides are converted to the acid during acid hydrolysis.

Table II

Automated Edman Degradation of Hb Deaconess βTpXIII

Residue #	Sequence #	I.D.	nmoles
1	121	Glu°	175.0
2	122	Phe	154.4
3	123	Thr	42.1
4	124	Pro	68.9
5	125	Pro	56.9
6	126	Val	63.0
7	127	Gln°	—
8	128	Ala	49.5
9	129	Ala	55.4
10	130	Tyr°	30.1
	Deletion of β131 (Gln)		
11	131	Lys*	5.2

The sample contained 320 nmoles of peptide.
° Identified as the trimethylsilyl deriative and confirmed by thin-layer chromatography. The value for the first residue was obtained from a separate experiment in which the peptide was not reacted with 4-sulfophenylisothiocyanate.
* Identification by back hydrolysis with hydriodic acid.

position 121 and residues of glutamine at positions 127 and 131. To determine which of these residues were deleted, the peptide was subjected to automated Edman degradation. The results are given in Table II. Both the glutamic acid at position 121 and glutamine at 127 were determined, suggesting that glutamine 131 was the deleted residue. However, the very low yield of lysine at cycle 11

and the absence of glutamine did not provide direct proof of the indicated deletion.

Table III provides kinetic data on the release of amino acids from the carboxyl-terminus of the peptide. The control experiment with normal βTpXIII shows that glutamine 131 is cleaved after two minutes of incubation. In Hb Deaconess βTpXIII, glutamine appears only after 15 minutes of incubation, and there is one residue after 30 minutes compared to two residues in the control. In addition, alanine is detected before glutamine, which is consistent with the data that shows glutamine 131 is deleted in Hb Deaconess.

Glutamine 131 is one of the residues in the H helix of the β-chain that is involved in the $\alpha_1\beta_1$ contact. Perutz et al (15) indicate that this is an area of larger contact between unlike

Table III

Digestion of βTpXIII from Hb Deaconess by Carboxypeptidase A & B

	Time (minutes)						
	2.0	30.0	0.5	1.0	2.0	15.0	30.0
Amino Acids	Control		Deaconess				
Lysine	1.0	1.0	1.1	1.0	1.0	1.0	1.0
Glutamine*	1.3	2.0	—	—	—	0.6	1.1
Tyrosine	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Alanine	0.4	1.5	—	—	0.4	1.2	1.6

* Glutamine elutes in the position of serine. Incubation was in 0.2M N-ethyl morpholine pH 8.5 at 37°C. The reaction was terminated by adding 1 drop of 6 N HCl and the mixture applied directly to the columns of the amino acid analyzer.

chains and that it undergoes a smaller shift during oxygenation and deoxygenation. Hemoglobins Khartoum, β124 (H2) Pro→Arg (16) and Yoshizuka, β108 (G10) Asn→Asp (17), which are mutants in the same contact area, produce no reported clinical symptoms in heterozygous individuals, except for a mild anemia in the case of individuals with Hb Yoshizuka. In this instance, the patient with Hb Deaconess has a borderline anemia (12 gm hemoglobin per 100 ml blood) but no other detectable clinical symptoms. Although Hb Deaconess shows some abnormal properties, it does not appear to impart any major disadvantages to the heterozygous individual.

** Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U. S. Department of Health, Education, and Welfare.

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