HEMOGLOBIN PRESBYTERIAN: β108 (G10) ASPARAGINE→LYSINE. A HEMOGLOBIN VARIANT WITH LOW OXYGEN AFFINITY

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1. Introduction

Characterization of abnormal hemoglobin variants continues to provide important information on the relationship between structural and functional properties of hemoglobin. Correlation of the properties of specific variants with the three-dimensional structure of hemoglobin has clarified which features of the molecule govern particular aspects of its properties and activity at the molecular level.

Hemoglobin Presbyterian is a new variant which migrates electrophoretically between Hbs A and F on cellulose acetate (pH 8.4), and in the Hb C position on citrate agar (pH 6.0). The variant was found in a 7-yr-old girl, her father, and paternal grandmother, all of whom have mild anemia. Although the variant hemoglobin exhibited low oxygen affinity, the effect of the allosteric effector 2,3-bisphosphoglycerate on the variant hemoglobin and on Hb A was similar. The degree of cooperativity was unchanged, and there was a slight increase in the Bohr effect. The low oxygen affinity can probably be explained on the basis of the disruption of the hydrogen bond between histidine α103 (G10) and the carbonyl of asparagine β108 (G10), which is now replaced by lysine in Hb Presbyterian. This report presents data on the structural and functional properties of this variant.

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2. Materials and methods

Electrophoresis and other techniques used in screening for hemoglobinopathies have previously been described [1]. Hemoglobins were separated by ion-exchange chromatography on DEAE-Sephadex [2], and globin chains were isolated by the Clegg procedure [3]. The abnormal β chain was reduced, aminoethylated [4], and digested with trypsin [5]. The digest was subjected to peptide fingerprinting [6] and to column chromatography on Aminex A-5 (Bio-Rad Laboratories) [7]. Peptides were further purified on Dowex 50 X 2 [8].

Peptides were hydrolyzed in 6 N HCl at 110°C for 24 h in vacuo and analyzed on a Beckman Model 121 amino acid analyzer by the method of Spackman et al. [9]. Sedimentation velocity determinations were performed in a Beckman Model E analytical ultracentrifuge as reported [10].

The oxygen equilibrium studies were done by the spectrophotometric method of Riggs and Wolbach [11]. Approximately 60 μM solutions in 0.05 M [*bis* (2-hydroxyethyl) amino] Tris(hydroxymethyl)-methane (Bistris) and Tris buffers were used. The abnormal hemoglobin was incubated at 20°C, and the allosteric effector was added in 100-fold molar excess over tetramer. Hb A, purified from the same column, was used as the control for all functional studies.

Hematological data were obtained by standard methods [12]. Enzymes were purchased from

Worthington Biochemical Corporation, and 2,3-bisphosphoglycerate was obtained from Sigma Chemical Co. All other chemicals were of analytical grade.

3. Results and discussion

Three generations of this family (the grandmother, her son, and one of his daughters) possess this abnormal hemoglobin. The hematologic findings in the grandmother are unremarkable, and no direct clinical disadvantages could be attributed to the presence of this variant. The isopropanol and heat tests for unstable hemoglobins were negative although the reticulocyte count was increased (3.5%). Quantitation of the hemolyzate gave 3.2% Hb A_2, 39.8% Hb Presbyterian, and 57% Hb A. The level of Hb F by the Singer method was 0.7%.

The variant hemoglobin migrates between Hbs A and F on cellulose acetate (pH 8.4) and at Hb C on citrate agar (pH 6.0). Globin chain analysis in alkaline buffer showed an abnormal β chain with a mobility like the βS chain. In acid buffer the abnormal chain migrated between the βA and βS chains, but closer to βA. Separation of chains by the Clegg procedure indicated an abnormal β chain with the gain of one net positive charge.

Peptide fingerprinting showed that βTp XIIa was absent (fig.1), and two new peptides were evident. After elution and amino acid analysis, these peptides were shown to be composed of residues 105–108 and 109–112. The single aspartic acid (asparagine) was missing, and lysine was detected as inclusive in residues 105–108.

Further evidence that asparagine 108 was replaced by lysine was obtained after quantitative recovery of peptides by column chromatography. Figure 2 shows the distribution of peptides. Residues 109–112 and βTp VIII, IX were purified by rechromatography of peak A (table 1). Also, peaks B and C were combined and rechromatographed (insert fig.1). Amino acid analysis of these peptides showed that peak I contained residues 109–112, and peak III was βTpI. Peak II contained residues 105–108 in which asparagine was replaced by lysine at position 108 in the β chain (table 1).

Figure 3 shows that Hb Presbyterian is a low affinity hemoglobin, with an observed right shift of

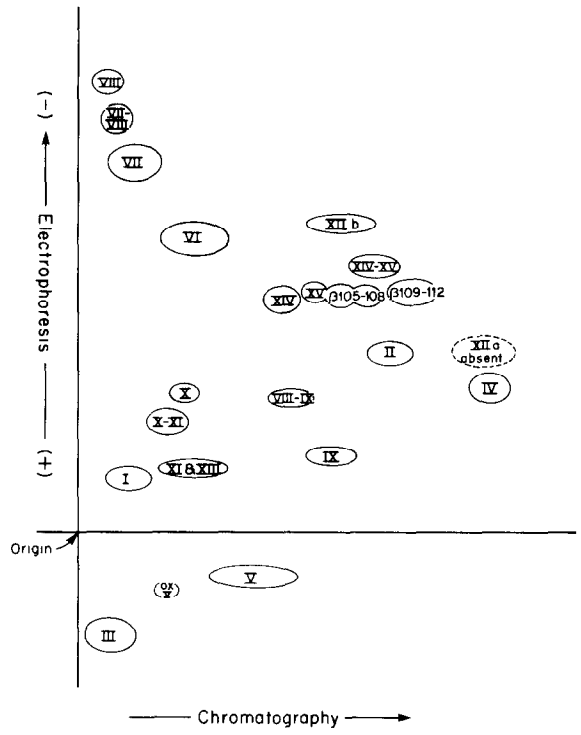

Fig.1. Peptide map of a tryptic digest of Hb Presbyterian. Chromatography was performed in isoamyl alcohol–pyridine–water (7:7:6, by vol.), and electrophoresis was carried out at pH 6.4.

the log $P_{½}$ vs. pH curve. The degree of cooperativity was similar to that of Hb A. The Bohr value ($\Delta \log P_{½}/\Delta$pH) was −0.62 for Hb A and −0.80 for Hb Presbyterian. The effect of 2,3-bisphosphoglycerate on Hb A and the variant hemoglobin was similar.

Asparagine β108 (G10) is at the $\alpha_1\beta_1$ contact, which is the most extensive contact between unlike chains, but where only slight movement occurs in the allosteric transition from oxy- to deoxyhemoglobin. Generally, mutations in this contact do not produce as pronounced functional changes as in the $\alpha_1\beta_2$ contact where movement is much greater as the two subunits slide pass each other. Unlike the $\alpha_1\beta_2$ contact, the $\alpha_1\beta_1$ contact is not closely connected to the heme group, and therefore changes in this contact are not usually reflected in changes in the heme environment.

The three-dimensional structure of hemoglobin shows that asparagine β108 (G10) is linked by a

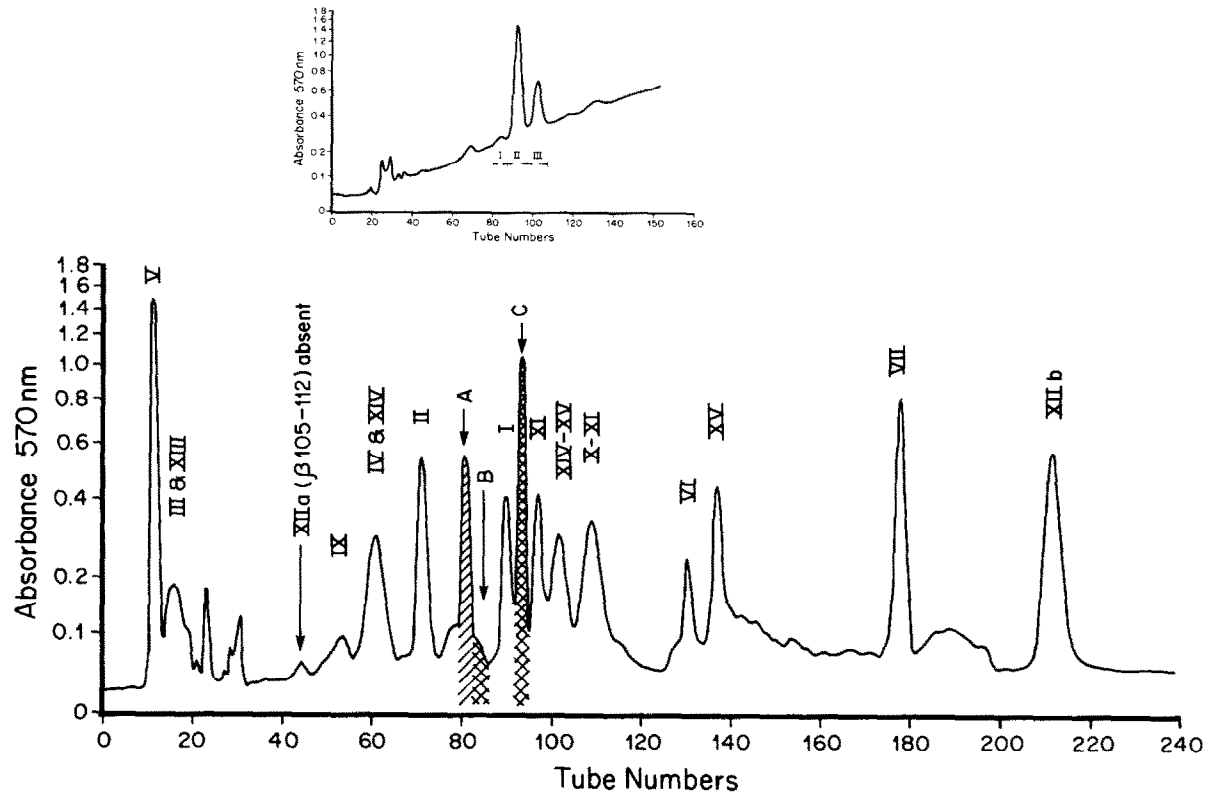

Fig.2. The separation of tryptic digest of Hb Presbyterian by column chromatography on Aminex A-5. The insert panel shows the purification of peaks B and C by rechromatography on Dowex 50 × 2. The ninhydrin product was monitored continuously at 570 nm.

Table 1
Amino acid composition of peptides from Hb Presbyterian

Amino acid	Peak A				Peaks B and C					
	β 109–112		β 66–82		I (β 109–112)		II (β 105–108)		III (β 1–8)	
	calcd	found	calcd	found	calcd	found	calcd	found	calcd	found
Lys			2	1.5				0.9	1	0.8
AE–Cys	1	0.7			1	0.4				
His			1	0.8					1	0.6
Asp			3	2.5			1	0		
Thr									1	0.8
Ser			1	1.0						
Glu									2	1.9
Pro									1	0.9
Gly			2	2.1			1	1.0		
Ala			2	1.8						
Val	2	1.7	1	1.0	2	1.6			1	0.7
Leu	1	1.0	4	4.0	1	1.0	2	2.0	1	1.0
Phe			1	0.9						

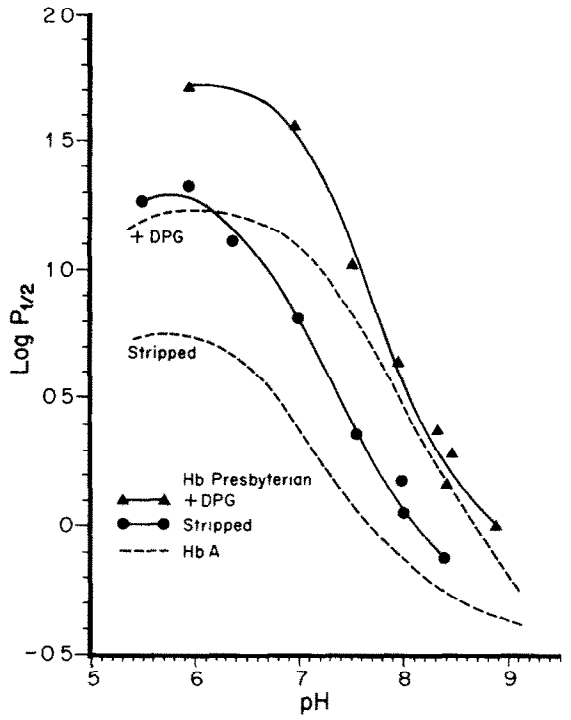

Fig.3. The effect of pH and organic phosphate on the oxygen affinity of Hb Presbyterian. The pH of the hemoglobin solution was measured after addition of the allosteric effector. Other experimental details are found in the text and in reference [10].

hydrogen bond to histidine α103 (G10) in the internal cavity [13]. This residue is also close to arginine β104 (G6) and lysine α99 (G6). In addition, asparagine β108 is linked extensively by hydrogen bonding through water molecules to other residues in both the α and β chains [14]. The substitution of asparagine by lysine probably results in the disruption of hydrogen bonds and electrostatic repulsion between residues. This could lead to rearrangement of the residues in the central cavity and procedure destabilization of the oxy conformation, which would be reflected in the observed low oxygen affinity of Hb Presbyterian.

Sedimentation velocity data indicate that substantial dissociation of tetramer to dimers does not occur with Hb Presbyterian under oxygenation conditions and that the low oxygen affinity is therefore not directly related to the degree of subunit dissociation. In contrast with Hb Kansas, a low affinity hemoglobin oxygenation is accompanied by a marked dissociation into subunits [15].

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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