HEMOGLOBIN $G_{Coushatta}$: A BETA VARIANT WITH A DELTA-LIKE SUBSTITUTION[1]

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Received January 16, 1967

Hemoglobin $G_{Coushatta}$ is an electrophoretically slow variant of human hemoglobin which was found to be inherited in several members of the Alabama-Coushatta Indian tribe who live on a reservation in Texas (Schneider, et al., 1964). Fingerprints and amino acid analysis revealed that in tryptic peptide β-III one of the two glutamic acid residues had been replaced by alanine in Hb $G_{Coushatta}$. The study described here indicates that the site of alteration in this hemoglobin variant is the 22nd residue from the N-terminus of the β chain. Alanine also occurs in the 22nd residue of the δ chain in the normally occurring minor component, Hb A_2, and in rare hemoglobins which are composed of junction products of both β and δ chains, i.e., the Lepore type hemoglobins (Baglioni, 1965). This latter class of hemoglobins appears to be the result of genetic events related to chromosomal crossing over. In Hb $G_{Coushatta}$, however, the amino acid substitution appears to be the result of a single transversion in the nucleotide triplet specifying the 22nd amino acid of the β chain.

Experimental Methods

Tryptic peptides of Hbs A and $G_{Coushatta}$ were compared by techniques described previously (Ingram, 1958; Naughton and Hagopian, 1962). Peptides β-II, β-III and β-V were purified by high voltage electrophoresis in varsol tanks containing volatile buffers of pH 6.5, 3.5 and 1.8. Descending chromatography with a pyridine : acetic acid : butanol : water solvent (100:30:150:120 v/v) was necessary for the purification of β-II. Amino acid analysis was carried out on a Spinco Model 120B automatic analyser with the following attachments: a model CRS-12AB Infotronics Integrator, high sensitivity cuvettes of 6.6 mm path length (Spinco part no. 3530034), and an expanded range card (Spinco part nos. 837662 and 327869). A 3 meter long extension was added to the tubing leading from the

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[1] The authors are indebted to Dr. Rose G. Schneider for providing the hemoglobin preparations investigated here. This work was supported by grants AM-08331 and HD-01963 from the N.I.H

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reaction bath to the colorimeter and was coiled in a 4 liter beaker filled with water. After high voltage electrophoresis, peptides were located on the ionogram by strip stains; they were removed with a razor blade and were sewed on a new piece of chromatography paper with a domestic sewing machine. After final electrophoresis, the peptides were eluted from the paper with 6 N HCl and hydrolyzed in evacuated hydrolysis tubes (Phoenix Precision Instrument Co., T-S 23-15) at 110° C for 22 hours. The hydrolysates were flash evaporated quickly at 40° C and redissolved in 0.2M sodium citrate buffer at pH 3.28 and subjected to amino acid analysis.

Peptide β-III was enzymatically hydrolyzed with elastase (Worthington) into smaller fragments. These were separated by electrophoresis at pH 6.5 and 3.5 (Hunt and Ingram, 1959).

Leucine amino peptidase digestion of peptide β-III was carried out by adding 0.05 mg of the enzyme (Worthington) to approximately 0.1 μ mole of peptide in 0.1 ml of water, 0.025 ml of 0.025 M $MgCl_2$ and 0.025 ml of 0.5 M Tris at pH 8.5. Aliquots were removed and mixed with 0.005 ml of glacial acetic acid after 30, 60, 90, 120, 150, 180 and 210 minutes of digestion. These were dried in vacuo and submitted to amino acid analysis.

Qualitative N-terminal analysis was performed by the method of Morse and Horecker (1966) on thin layer plates of Silicagel G. The chromatography solvent employed was chloroform : ethanol : acetic acid (38:4:3 v/v). The plates were examined under a long wave ultra violet lamp. Positions of the Dansyl amino acid derivatives of peptides β-II and β-III were compared to authentic Dansyl amino acids on the same chromatography plate.

Results and Discussion

Fingerprints of tryptic peptides of Hb $G_{Coushatta}$ revealed an altered electrophoretic migration of β-III (Fig.). The slower anodal migration of this peptide mimics the slower migration of the intact hemoglobin during electrophoresis at alkaline pH. This was particularly obvious after the fingerprint had been stained with Sakaguchi's stain. Amino acid analysis of β-III established a substitution of alanine for glutamic acid (Table 1). There were two possibilities for the site of substitution, β^{22} and β^{26} (Braunitzer, et al., 1960). Analyses of elastase fragments a and b indicated that the substitution was located in residue 22 of the β chain (Table 2). This was substantiated by digestion of β-III from $G_{Coushatta}$ with leucine amino peptidase (Table 3). This enzyme liberated valine, asparagine, aspartic acid and alanine initially from β-III in Hb $G_{Coushatta}$, whereas in β-III from Hb A valine, asparagine, aspartic acid and glutamic acid were liberated during the first period of digestion. Because β^{22} was one of the amino acid differences found when comparing the β and δ chains (Ingram and Stretton, 1962), it was necessary to examine other pertinent sequences of the non-α chain of Hb $G_{Coushatta}$ to determine whether or not this variant

Table 1

Amino Acid Composition of Tryptic Peptides β-3 from Hemoglobins A and $G_{Coushatta}$

	β-3 from Hb $G_{Coushatta}$		β-3 from Hb A	
Amino acids	Micromoles x 10^{-2}	Residues	Micromoles x 10^{-2}	Residues
Lysine	0.009	-	0.008	-
Histidine	0.014	-	0.011	-
Arginine	0.307	1.0	0.286	0.9
Aspartic Acid	0.610	1.8	0.608	1.9
Threonine	0.007	-	0.010	-
Serine	0.008	-	0.013	-
Glutamic Acid	0.410	1.2	0.628	2.0
Proline	0.017	-	0.023	-
Glycine	0.980	2.8	0.871	2.7
Alanine	0.727	2.1	0.343	1.1
Valine	0.919	2.7	0.859	2.7
Isoleucine	-	-	0.006	-
Leucine	0.344	1.0	0.318	1.0

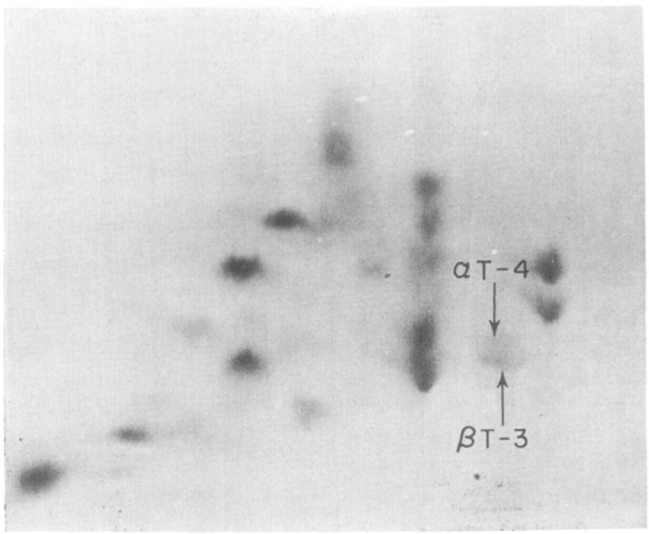

Fig. Fingerprint of Hb $G_{Coushatta}$. Arrows point to migration of the altered peptide (βT-3) in relation to αT-4.

Table 2

Amino Acid Composition of Two Elastase Fragments of Tryptic Peptide β-III from Hb $G_{Coushatta}$

Micromoles x 10^{-2}

Amino Acid	a	b
Glutamic Acid	0.251	0.132
Glycine	0.509	0.292
Alanine	-	0.143
	(Gly Gly Glu)	(Gly Gly Glu Ala)

Table 3

Amino Acids Liberated by LAP Digestion of β-III of $G_{Coushatta}$ (Micromoles x 10^{-2})

-Val · Asn · Val · Asp · (Glu or Ala) · Val · Gly · Gly · (Glu or Ala) · Ala · Leu · Gly · Arg-

	β-III Hb $G_{Coushatta}$								β-III Hb A		
Digestion Time (minutes	5	30	60	90	120	150	180	210	60	150	330
Aspartic Acid	-	.100	.220	.359	.405	.512	.563	.784	.055	.140	.130
Asparagine	.030	.387	.381	.317	.317	.312	.322	.354	.178	.193	.128
Glutamic Acid	-	-	.044	.129	.235	.233	.345	.582	.019	.085	.078
Glycine	-	.027	.133	.323	.531	.698	.999	1.506	.023	.094	.136
Alanine	-	.069	.287	.466	.553	.723	.861	1.208	-	.028	.042
Valine	.045	1.159	1.188	1.316	1.357	1.525	1.509	1.890	.579	.705	.408
Leucine	-	-	.067	.097	.309	.235	.354	.554	N.T.	N.T.	N.T.

Table 4

Amino Acid Composition of Tryptic Peptides β-II and β-V of Hb $G_{Coushatta}$

	β-II			β-V		
	Micromoles	Residues	Expected if β-II	Micromoles	Residues	Expected if β-V
Lysine	0.141	1.0	1	0.563	1.0	1
Aspartic Acid	0.011	0.1	1	1.461	2.6	3
Threonine	0.151	1.1	1	0.563	1.0	0
Serine	0.170	1.2	0	0.903	1.6	3
Glutamic Acid	0.030	0.2	0	0.600	1.1	1
Proline	-	-	0	1.176	2.1	2
Glycine*	0.176	1.3	1	1.188	2.1	2
Alanine	0.227	1.6	2	0.663	1.2	1
Valine	0.159	1.1	1	0.621	1.1	1
Methionine	0.000	0.0	0	0.518	0.9	1
Leucine	0.160	1.1	1	0.574	1.0	1
Phenylalanine	-	-	0	1.698	3.0	3
Tryptophane	Positive Ehrlich Stain		1	-	-	-
N-Terminal	Serine		Threonine			

was a Lepore hemoglobin. The second and fifth tryptic peptides of the non-α chain demonstrate sequences characteristic of either the β or δ chains, and are located toward the N- and C- terminal sides, respectively of β^{22}. Therefore, these two peptides were examined in Hb $G_{Coushatta}$ and were both found to be characteristic of the β polypeptide chain (Table 4). In Hb $G_{Coushatta}$ serine was the N-terminal amino acid of β-II and aspartic acid was absent. In the δ chain threonine is the N-terminal amino acid and aspartic acid is present in the second tryptic peptide. Accordingly, β-V also failed to show characteristics of being δ-like; one residue of threonine and two residues of serine were present. If it had been δ-like, there would have been three residues of serine and no threonine present. Therefore, since Hb G_{Cousha} is β-like on both sides of the substitution, β^{22}, this variant is most likely the result of a single alteration (A→C) in the nucleotide triplet (Brimacombe, et al., 1965) specifying the 22nd residue of the β chain. There is, however, no way of ruling out the possibility that this hemoglobin variant could have been the result of a double crossing-over event occuring between the structural genes specifying the β and δ polypeptide chains. Up to this time, there have been no other hemoglobin variants reported which have a single δ-like alteration.

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