THE AMINO ACID COMPOSITION OF HGB NEW HAVEN #2

($HGB\ N_{New\ Haven}$)*

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Received May 26, 1964

Hgb New Haven #2 (NH-2), detected in a Caucasian family of French extraction, has been characterized electrophoretically and chromatographically. Fingerprints of the tryptic peptides of the isolated abnormal hemoglobin have been performed. The amino acid substitution has been identified by quantitative amino acid analysis as gly ⟶ asp at the 16th residue from the $-NH_2$ terminus of the β chain. Hgb New Haven #2 is, therefore, $\alpha_2\beta_2^{16\ asp}$.

Case History: Electrophoresis of the hemoglobins from the patient, F. L., a healthy obese Frenchman, age 56 years, was performed as part of a family study related to the investigation of a non-thalassemic, refractory, hypochromic microcytic anemia in his daughter, Mrs. H. D. Although

*This investigation was supported by USPHS Grant No. A2956 from the National Institutes of Health, Bethesda, Maryland, and Grant HE07616-02 from the U.S. Public Health Service.

no hemoglobin abnormalities were noted in the erythrocytes from Mrs. H. D., her father's hemolysate revealed a rapidly migrating component which we have designated Hgb New Haven #2. Hematologic data in F. L. were within normal limits except for a reticulocytosis of 4% and a MCV of 100 cu. microns. A comprehensive report of the hematologic findings and genetic constitution of this family will be published in the near future.

Electrophoretic properties: Hgb New Haven #2 migrated on paper at pH 8.6 in barbital buffer, 0.06 ionic strength, with a mobility faster than Hgbs J and K but slightly slower than Hgbs H, I and R (Durham #1). Hgb NH-2 is presumed to migrate in the approximate position of Hgbs N, Hopkins No. 2, Norfolk and Mexico although samples were not available for comparison. Incomplete separation from Hgb A, analagous to the findings with Hgbs J and K, was noted on paper electrophoresis at pH 6.5 using a 0.1 M phosphate buffer. Starch gel electrophoresis utilizing Tris-Borate-EDTA buffer at pH 8.5 (Chernoff, et. al. 1964) clearly distinguished NH-2 from the faster moving hemoglobins, H, I and R, as well as from the slower moving compounds, J and K.

Chromatographic properties: Separation of the hemolysate on CMC (Chernoff, et. al., 1964) revealed that Hgb New Haven #2 eluted from the cellulose 0.15 pH units before the major fraction of normal hemoglobin, Hgb A_0. Separation on DEAE cellulose (Chernoff, et. al. 1964) indicated that NH-2 was eluted from the column at a phosphate molarity of 0.0095, in contrast to Hgb A which elutes in this system at a phosphate molarity of 0.006. Hgb NH-2 comprised approximately 40% of the total hemoglobin mass by DEAE chromatography.

Separation of the polypeptide chains of globin New Haven #2 in urea veronal starch gel electrophoresis (Chernoff and Pettit, 1964) indicated the

abnormality to be localized to the β chain. The abnormal β chains of NH-2 migrated more rapidly toward the anode than normal β chains but somewhat more slowly than the abnormal β chains of Hgb R.

Peptide analysis: Fingerprints of the tryptic peptides (Chernoff and Liu, 1961) of globin of purified New Haven #2 revealed that peptide β T II, easily identified by staining procedures because it contains tryptophane, was displaced from its normal position. The abnormality in the position of β T II was even more strikingly demonstrated when the tryptic digest of isolated β chains of NH-2 were fingerprinted (Chernoff, 1964a). In addition, peptide maps of the β chain of Hgb New Haven #2 revealed no other peptides displaced from their normal positions or with abnormal staining characteristics.

Isolation of β T II was carried out by chromatographic means on Dowex 1-X2 (Chernoff, 1964b) followed by separation of selected portions of the Dowex 1 effluent on the short column resin (Type 15A) of the Spinco Amino Acid Analyzer system by the method of R. T. Jones (1962, 1964). Quantitative amino acid analysis, performed on an automatic amino acid analyzer by the method of Moore, et. al. (1958) revealed that one residue of glycine was absent while a single residue of aspartic acid was present. In view of the nature of the composition of β T II, the amino acid defect could be placed at position 16, barring the unlikely possibility of additional mutational events, such as inversions or fortuitous multiple amino acid replacements. Further proof of the positioning of the abnormality was obtained by controlled dilute acid hydrolysis (0.03 N HCl) which cleaves polypeptides at aspartyl residues and releases free aspartic acid into the medium 16 hours hydrolysates at 108° in sealed evacuated tubes of β T II from NH-2 in 0.03 N HCl freed not only aspartic acid but also lysine, providing conclusive proof that the substitution occurred at the penultimate amino acid from the -COOH terminus of

TABLE I

Amino Acid Residues Per Molecule of βT II*

	βT II (Hgb A)		βT II (Hgb NH-2)	
	Observed	Integral	Observed	Integral
Lysine	1.0	1	.78	1
Aspartic Acid	.09		1.09	1
Threonine	.96	1	1.0	1
Serine	.67	1	.91	1
Glycine	1.1	1	.22	
Alanine	2.1	2	1.98	2
Valine	1.1	1	1.09	1
Leucine	1.03	1	1.06	1
Tryptophane+	+		+	

*24 hr. hydrolysis, 6 N HCl 108°

+Try present by specific stain in fingerprints.

βT II (Hgb A)	Ser-Ala-Val-Thr-Ala-Leu-Try-Gly-Lys
βT II (Hgb NH-2)	Ser-Ala-Val-Thr-Ala-Leu-Try-Asp-Lys

this tryptic peptide. Although we have not yet determined whether aspartic acid or asparagine is present in position 16, the electrophoretic characteristics of Hgb New Haven #2 strongly suggest that this residue is in the form of aspartic acid.

Although a number of hemoglobins have been described migrating electrophoretically in a manner similar to that of Hgb New Haven #2 (i.e., Hgbs N, Norfolk, Mexico, etc.) none of these has yet been identified as

$\alpha_2\beta_2{}^{16\ asp}$. Hgb Norfolk has been shown to have the formula $\alpha_2{}^{57\ asp}\beta_2$ (Baglioni, 1962) while Hgb Mexico is described as $\alpha_2{}^{54\ glu}\beta_2$ (Jones, et. al., 1963). We propose, therefore, that other abnormal hemoglobins found to have the same amino acid substitution as New Haven #2 be referred to by the designation Hgb $N_{New\ Haven}$.

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