HEMOGLOBIN RIVERDALE-BRONX AN UNSTABLE HEMOGLOBIN RESULTING FROM THE SUBSTITUTION OF ARGININE FOR GLYCINE AT HELICAL RESIDUE B6 OF THE β POLYPEPTIDE CHAIN.

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Summary

Hemoglobin Riverdale-Bronx is a heat unstable hemoglobin isolated from the erythrocytes of a 45 year old woman of German-Jewish ancestry who had a fairly well compensated hemolytic anemia. Structural studies of this hemoglobin have revealed the substitution of arginine for the normally occurring glycine at helical residue B6 (β24) of the β polypeptide chains. According to Perutz, Kendrew and Watson, glycine B6 is an invariant non-polar residue, which is in close contact with E8, a glycine residue adjacent to the distal heme-linked histidine (E7). The replacement of the invariant non-polar glycine with arginine at this site would be expected to alter the relationships of the E-helix to the heme of the affected (β) polypeptide chains.

Several abnormal human hemoglobins which are associated with accelerated erythrocyte destruction *in vivo* and with precipitation of the hemoglobin (Hb) on warming to 50° *in vitro* have been encountered. Most of these unstable hemoglobins result from amino acid substitutions in the β or α (more commonly β) polypeptide chains, although two, Hb Freiburg[1] and Hb Gun Hill[2] result from deletions. Perutz and Lehmann in a recent review[3] pointed out that the substitutions (or deletions) in many of these unstable hemoglobins result in alterations in the pattern of non-polar contacts, particularly those adjacent to the heme groups, or in changes in helical structure of the polypeptide chain

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accompanying replacement of or replacement with a proline residue. The present report is concerned with another example of an unstable hemoglobin, designated Hemoglobin Riverdale-Bronx (Hb RB), found in a 45 year old Jewish woman at the Mount Sinai Hospital in New York City.

Methods

Vertical starch gel electrophoresis was carried out at pH 8.6 with Tris-EDTA-Borate buffers (Fig. 1). Hybridization with known α and β chain variants of human hemoglobin was done by the methods described previously[4]. Starch granule electrophoresis was carried out by the method of Kunkel et al[5] with minor modifications.

Hemoglobin RB was isolated by electrophoresis on starch granules, or by chromatography on microgranular DEAE-cellulose (Whatman DE-52) using Tris-HCl buffers. Structural studies were carried out on two separate samples of hemoglobin RB, one separated electrophoretically and the other chromatographically. Globin was prepared by precipitation with acid-acetone at -20°. The α and β polypeptide chains of Hb RB were separated by chromatography of globin on carboxymethylcellulose with 8M urea and mercaptoethanol by the methods of Clegg et al[6]. (Aminoethylation was omitted in the initial runs in order to reduce the number of peptides and thus diminish the possibilities of contamination with other peptides on elution from fingerprints.) Twelve mgs. of β globin from Hb RB was digested with trypsin (Worthington, twice crystallized) and following lyophilization, maps of the soluble peptides of Hb β^{RB} and of Hb H (β_4^A) were prepared by electrophoresis for 1-3/4 hours, followed by chromatography. Methods and solvents for peptide mapping were those of Clegg et al[6] except that ascending chromatography was employed. Peptides to be analysed were eluted from four peptide maps by the methods of Sanger and Tuppy[7], and hydrolyzed at 105° in sealed capillary tubes for 18 hours. The contents of the capillary tubes plus distilled water rinsings were lyophilized and dissolved in 0.2N citrate buffer pH 2.2 for amino acid analysis in a Beckman amino acid analyzer, Model 120B.

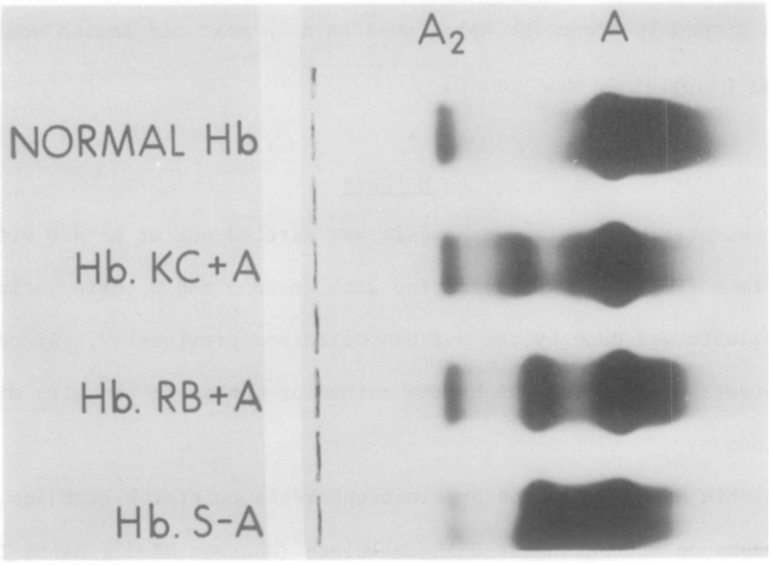

Figure 1: Vertical starch gel electrophoretic pattern of Hb RB (third sample from top). Tris-EDTA-Borate buffer pH 8.6. Anode on right. Benzidine stain. (Hb KC is another unstable hemoglobin currently under study.) Note that Hb RB has approximately the same mobility as Hb S (fourth sample).

Results

Hemoglobin Riverdale-Bronx was more basic than hemoglobin A and its migration in alkaline buffers resembled that of hemoglobin S (Fig. 1). Hemoglobin RB accounted for 30 per cent of the hemoglobin when separation was accomplished by electrophoresis on starch granules in 0.05M barbital buffer pH 8.6. Recombination of this hemoglobin at pH 4.7 with isolated hemoglobin I (an α chain variant) resulted in the appearance of two 'new' hemoglobins; the abnormality therefore appeared to be in the β polypeptide chains.

Peptide maps of the β chains of Hb RB and of normal β chains (of Hb H) revealed the disappearance of normal tryptic peptide 3 (residues 18-30) in Hb RB and the appearance of two peptides not seen on the maps of normal β chains. The

TABLE I

Amino Acid Composition of Tryptic Peptides β3A and β3B of Hemoglobin Riverdale-Bronx and of Normal βT3.

Amino Acid	Number of residues			
	Expected βT3 Normal	Found βT3 Hb H	Found βT3A Hb RB	Found βT3B Hb RB
Arg	1.0	1.1	0.9	1.0
Asp	2.0	2.0	2.0	0
Glu	2.0	2.0	1.0	1.1
Gly	3.0	3.0	-	1.8
Ala	1.0	1.0	-	1.1
Val	3.0	2.9	3.1	-
Leu	1.0	1.0	-	1.1

two new peptides, designated βT3A and βT3B as well as normal peptide βT3 of Hb H were arginine positive on specific staining[8]. The amino acid analyses of these peptides are shown in Table I. The amino acid sequence of normal peptide βT3 is:

Val-Asn-Val-Asp-Glu-Val-Gly-Gly-Glu-Ala-Leu-Gly-Arg

Residue No.:	18	19	20	21	22	23	24	25	26	27	28	29	30
Helical Notation:	A15	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12

The amino acid composition of β chain peptides 3A and 3B of Hb RB indicated the substitution of arginine for glycine at residue 24 (helical residue B6) of the β chain in this abnormal hemoglobin which would result in the following sequence:

Val-Asn-Val-Asp-Glu-Val-Arg ¦ Gly-Glu-Ala-Leu-Gly-Arg

Residue No.: 18 24 30

Tryptic digestion of β chains with an arginine residue at β^{24} resulted in a cleavage indicated by the vertical line at the 'new' arginine residue to yield peptides 3A (residues 18-24) and 3B (residues 25-30).

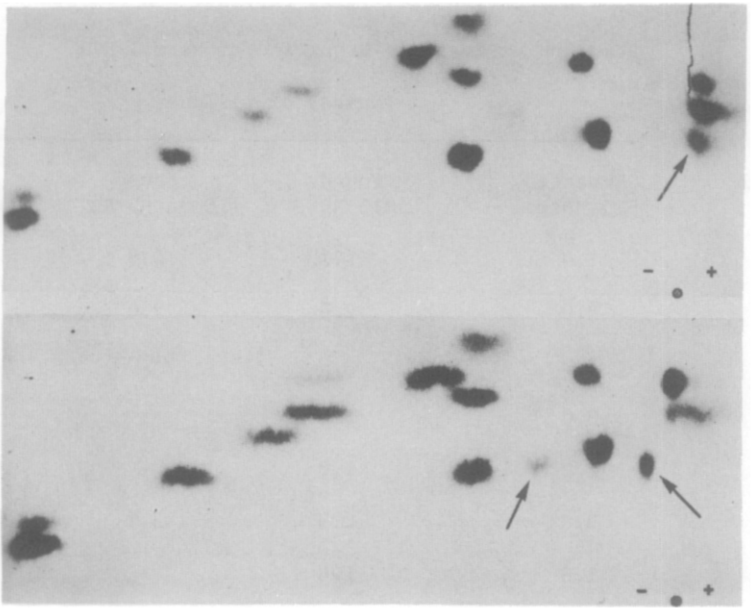

Figure 2: Peptide maps of tryptic digest of β^A chains (upper) and β chains of Hb RB (lower). Peptide βT3 in normal β chains is indicated by an arrow. In β chains of Hb RB, two 'new' peptides are indicated by arrows: βT3A on right and βT3B on left.

Discussion

Helical residue B6, the site of the substitution of arginine for glycine in Hb RB, is an "invariant" residue, since it has been found to be glycine in all normal myoglobins and hemoglobins thus far examined[9]. According to Perutz, Kendrew and Watson[9] the normally occurring glycine at β6 is in close contact with the glycine of βE8, and B6 is one of the interior sites lacking contact with the surrounding water. Most of these interior sites including B6 are occupied by non-polar residues in normal hemoglobin. The close relationship of B6 and E8 may be appreciated from Dickerson's two dimensional representation of myoglobin (Fig. 3)[10]. The substitution of the larger and polar arginine residue for glycine at the

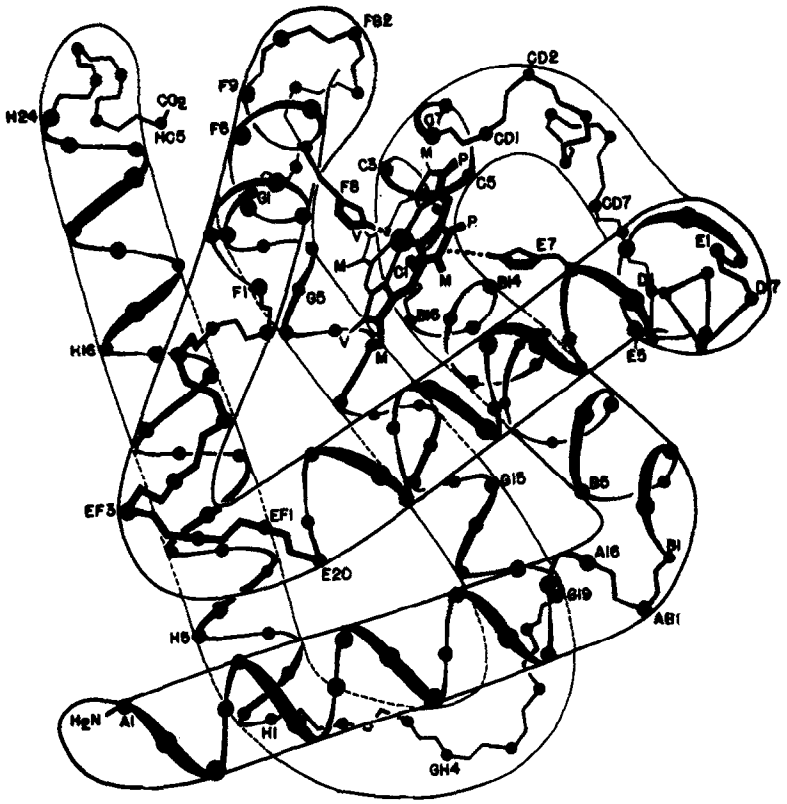

Figure 3: α-carbon diagram of myoglobin molecule from R.E. Dickerson. (Reproduced by permission of the author and Academic Press.[10])

structurally important non-polar site B6 would be expected to affect the tertiary structure of the polypeptide chain. (The replacement of glycine by arginine need not introduce an internal polar group; as in Hb Sogn, in which leucine A11β is replaced by arginine, the guanidinium group might protrude on the surface[3].) This substitution would however probably lead to considerable distortion of the E-helix as well as of the β-helix and thus of the relationships of the heme to the adjacent polypeptide chain. The distal heme-linked histidine is the seventh residue of the E-helix and of the approximately 60 contacts between the heme and its polypeptide chain, at least five are with residues in the E-helix, according to Perutz et al[11]. No loss of heme groups was demonstrable in Hb RB: the ratio of heme to globin[2] was within the range observed in normal human hemoglobin. In

preliminary electron paramagnetic resonance studies marked differences have been observed between methemoglobin RB and normal methemoglobin[12]. These differences probably reflect the altered relationships of the β heme groups and their polypeptide chains in Hb RB.

The patient in whom Hb RB was found had a mild hemolytic anemia (hemoglobin 11-12 grams per cent, reticulocytosis 10 per cent) with splenomegaly, but without pigmenturia. She had no children and her parents were dead. The findings associated with Hb RB, together with the observation that the same substitution, arginine for glycine, one residue removed, at B7 occurred in apparently healthy individuals (Hb G-Taiwan[13]), provide further evidence for the importance of the invariant glycine B6 in the stability of the hemoglobin molecule. It seems reasonable to consider hemoglobin RB as one of the unstable hemoglobins resulting from an amino acid substitution which alters the relationships of the heme group to its polypeptide chain.

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