Comparative Studies of Hb Lepore Boston, Hb A\textsubscript{2}, and Hb A\textsuperscript{*}

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KAZUHIKO ADACHI, TOSHIO ASAKURA, FRANCES M. GILL, AND ELIAS SCHWARTZ
From the Division of Hematology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania
19104

Several functional tests were performed to compare Hb A, Hb A\textsubscript{2}, and Hb Lepore Boston, which has a \(\delta\)-\(\beta\) crossover in the region of residues 87 to 116. Oxygen equilibrium curves determined by an automatic apparatus in 0.1 m potassium phosphate buffer, pH 7.0, at 20\(^\circ\) showed that the \(p_{50}\) was 5.8 mm Hg for Hb Lepore Boston, in contrast to 8.1 and 10.3 mm Hg for Hb A\textsubscript{2} and Hb A, respectively. The \(n\) values (Hill coefficients) of Hb Lepore Boston and Hb A\textsubscript{2} were slightly smaller than that of Hb A. The effect of 2,3-diphosphoglycerate and inositolhexophosphate on the \(p_{50}\) of Hb Lepore Boston and Hb A\textsubscript{2} was less than that on the \(p_{50}\) of Hb A. The molecular stability to mechanical shaking of Hb Lepore Boston and Hb A\textsubscript{2} showed that the oxy forms of Hb Lepore Boston and Hb A\textsubscript{2} denatured at a rate 3 times faster than that of Hb A. MetHb Lepore Boston was more unstable than MetHb A\textsubscript{2} to mechanical shaking. These results indicate that, although the molecular stability of Hb Lepore Boston is more similar to that of Hb A, than that of Hb A, the oxygen-binding properties of Hb Lepore differ from both Hb A and Hb A\textsubscript{2}.

Hb Lepore refers to a group of abnormal hemoglobins which probably arose by unequal crossover between the structural genes coding for the \(\delta\)- and \(\beta\)-polypeptide chains of human hemoglobin (1). The abnormal globin chain is 146 amino acids long with a \(\delta\) chain at the NH\(_{2}\)-terminal and a \(\beta\) chain sequence at the COOH-terminal. The region of crossover between \(\delta\) and \(\beta\) chain sequences differs in the three types of Hb Lepore which have been described (1-8).

We have studied Hb Lepore from a 41-year-old black male with a clinically mild sickling disorder who is heterozygous for Hb S and Hb Lepore. Amino acid analysis of the non-\(\alpha\) chain of Hb Lepore showed that the crossover was in the region of residues 87 to 116, the same as that found in Hb Lepore Boston (1-6). A previous report showed that Hb Lepore Boston and Hb Lepore Hollandia (\(\alpha\)\(_{2}\)(\(\delta\)\(_{2}\))\(\beta\)\(_{2}\)) have higher oxygen affinities than Hb A (9), but detailed analysis of the oxygen equilibrium curves and of the effect of organic phosphates on the oxygen-binding properties of Hb Lepore have not been reported. In the present studies, we determined the oxygen-binding properties of Hb Lepore Boston by the use of an automatic apparatus and compared them with those of Hb A and Hb A\textsubscript{2}. In addition, we have compared the stability to mechanical shaking of Hb Lepore Boston, Hb A, and Hb A\textsubscript{2}.

RESULTS

Oxygen Equilibrium Curves—The oxygen equilibrium curves of purified Hb Lepore Boston, Hb A, and Hb A\textsubscript{2} were determined at 20\(^\circ\) in 0.1 m potassium phosphate buffer, pH 7.0, in the presence and absence of organic phosphates.

Hb Lepore Boston showed a left-shifted curve. The \(p_{50}\) values of Hb Lepore Boston, Hb A\textsubscript{2}, and Hb A were 5.8, 8.1, and 10.3 mm Hg, respectively. The Hill plots of the curves are shown\(^1\) in Fig. 1. The Hill coefficients (\(n\) values) of Hb Lepore Boston and Hb A\textsubscript{2} were slightly lower than that of Hb A (Table I). The addition of 2 mM 2,3-DPG increased the \(p_{50}\) value of hemoglobins more than 3-fold (Fig. 1C, Table I). The \(\Delta F\) values for Hb Lepore Boston are clearly smaller than those for Hb A and Hb A\textsubscript{2}. Further analysis of the oxygen binding curves of these hemoglobins was done by calculating \(K\) and \(K_{r}\) values, the microassociation constants of the Adair equation of the first and last oxygen-binding steps (10). According to allosteric theory, high cooperativity can only be observed upon a complete transition from the T to the R states during ligation. Thus, in highly cooperative hemoglobins the bottom extreme end of the equilibrium curve (plotted as a Hill plot) will correspond to the oxygenation of the T structure (10, 11). As shown in Fig. 1A and Table II, the top extreme end of the oxygen equilibrium curves or \(K_{r}\) values of these three hemoglobins are the same. In contrast, the \(K\) values show significant differences among the three hemoglobins.

Mechanical Instability—The rate of precipitation of hemoglobin during mechanical shaking depends upon protein conformation in solution (12, 13). The oxy forms of Hb A\textsubscript{2} and Hb Lepore Boston precipitated 3 times faster than that of Hb A as shown in Fig. 2. The rates of precipitation of oxy, deoxy, and carboxy forms of Hb Lepore Boston and Hb A\textsubscript{2} were the

\(^1\) Portions of this paper (including "Materials and Methods," Figs. 1 and 2 and Tables I and II) are presented in a miniprint at the end of this paper. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 77M-1185, cite author(s), and include a check or money order for $1.20 per set of photocopies.

\(^*\) The abbreviations used are: 2,3-DPG, 2,3-diphosphoglycerate; IHP, inositolhexophosphate.
same. The carboxy forms of Hb A and Hb Lepore precipitated 1.8 times faster than that of Hb A. Eight per cent of the initial concentration of the deoxy forms of Hb A and Hb Lepore Boston precipitated after mechanical shaking for 30 min, while deoxy-Hb A did not precipitate at all in that length of time. The Met forms of Hb A and Hb Lepore Boston precipitated 1.5 and 2 times faster than that of Hb A, respectively. Thus, except for the Met form, Hb Lepore Boston precipitates at the same rate as Hb A, suggesting a similarity in their molecular conformation in solution.

**DISCUSSION**

Molecular Stability of Hb A and Hb Lepore – The oxy, carboxy, and deoxy forms of Hb Lepore Boston precipitate at rates similar to that of Hb A, but 3 times faster than that of Hb A. The similarity of the molecular stabilities of Hb A and Hb Lepore Boston may be related to the similarity of the first two-thirds of their amino acid sequence. Hb A and Hb Lepore differ by 10 amino acids, those at the positions of 9, 12, 22, 50, 66, 87, 116, 117, 124, and 126. The non-α chain of Hb Lepore Boston has an amino acid sequence which is the same as that of β chain from the NH2-terminal to position 115. Hb A and Hb Lepore Boston differ by only four amino acids, those at positions 116, 117, 124, and 126. The amino acid side groups at positions 9 (A6), 22 (B4), 50 (D1), 87 (F3), and 126 (H4) are situated on the external side of the molecule, while those of 12 (A9), 86 (F2), 116 (G18), 117 (G9), and 124 (H2) are located on the internal side (14). The substitutions of amino acids in the external positions from hydrophilic to hydrophobic groups appear to affect the mechanical stability of hemoglobin. For instance, the substitution of glutamic acid with a hydrophilic side chain by valine with a hydrophobic side chain at the 6th position of the β chain (A4) in sickle hemoglobin results in marked destabilization (15). The change of the glutamic acid at the 22nd position (B4) of the β chain to alanine in Hb A and Hb Lepore Boston may account for the relative instability of Hb A and Hb Lepore Boston when compared to Hb A. The slight difference in stabilities between MetHb Lepore Boston and MetHb A may be related to the substitution of amino acids at the 116 (G18) and 124 (H2) positions of the β chain, which are in contact with the α chain.

Functional Properties of Hb A and Hb Lepore Boston – Eddison et al. (16) reported that Hb Lepore had an increased oxygen affinity with a normal heme-heme interaction. More recently, McDonald et al. (9) found that Hb Lepore Boston and Hb Lepore Hollandia have higher oxygen affinities than does Hb A, with normal Hill coefficients and Bohr effects. No study has been done on the effect of organic phosphates on oxygen binding of Hb Lepore. Using a sensitive automatic continuous recording apparatus in the present study, the previous finding of increased oxygen affinity of Hb Lepore Boston was confirmed. In contrast to the previous study, we found that the $n_{\text{max}}$ (the maximum slope of the Hill plot) of Hb Lepore Boston is slightly decreased compared to that of Hb A. The total energy of interaction with oxygen for Hb Lepore Boston was also decreased. The oxygen equilibrium curve of Hb Lepore Boston is shifted to the right upon the addition of DPG and IHP, although the effects are not as great as the right shift observed in Hb A. Huisman et al. (17) reported that Hb A has a considerably higher oxygen affinity than Hb A, while Eddison et al. (16) and DeBrun and Jassan (18) found that Hb A and Hb A exhibited the same oxygen affinities. DeBrun and Jassan (18) also reported that DPG had an identical affect on the oxygen binding curves of Hb A and Hb A. The present study shows that the $p_{50}$ value of Hb A is slightly lower than that of Hb A, and that the organic phosphates DPG and IHP have a smaller effect on the oxygen binding of Hb A than on Hb A.

Comparison of the oxygen-binding properties of Hb Lepore Boston with those of Hb A and Hb A* clearly indicates that the functional properties of Hb Lepore Boston are not intermediate between Hb A and Hb A*, since the oxygen affinity of Hb Lepore Boston is higher than that of the other two hemoglobins. McDonald et al. (9) reported that the oxygen affinities of Hb Lepore Boston and Hb Lepore Hollandia were the same, and therefore concluded that the positions 50, 86, and 87 of the β or δ chain are not involved in the differences in oxygen affinities between Hb A and Hb Lepore Boston. They also suggested that the similarity in the functional properties of the two types of Hb Lepore strengthens the argument that the critical residues are the positions in the A and H regions (9). It is generally accepted that the oxygen affinity of hemoglobin is affected by the substitution of amino acids at the α and β contact region and by the replacements near the heme (19). Among 10 amino acid substitutions between Hb A and Hb A*, the positions of 116 and 124 are the amino acids at the contact region between the α and β chain. Although positions 116 and 124 are the same in Hb Lepore Boston and Hb A, the oxygen-binding properties of Hb Lepore Boston are much more similar to Hb A. Thus, it is not a simple matter to correlate the number and position of amino acid replacements with the oxygen-binding properties of hemoglobin. For example, the difference in the functional properties of Hb A and Hb A*, which differ by 10 amino acids, is smaller than that between Hb A and Hb Lepore Boston, which differ by only 6 amino acids.

The $K_{\text{d}}/K_{\text{a}}$ ratio of Hb Lepore Boston is about half that of Hb A and Hb A* (Table II). Those differences suggest that the fine structure of the tertiary and quaternary conformations of deoxy-Hb Lepore Boston is different from that of the other two hemoglobins. The effect of DPG and IHP on these hemoglobins is also different. The values of $K_{d}$ and the $K_{d}/K_{a}$ ratio of Hb Lepore Boston with or without organic phosphates are the smallest of the three hemoglobins. Neither the $K_{d}$ nor $K_{a}$ value of Hb Lepore Boston is greatly affected by organic phosphates, suggesting either that the conformations of the oxy and the deoxy forms of Hb Lepore Boston are not altered by binding with organic phosphates or that the affinity of organic phosphates for Hb Lepore Boston is less than that of Hb A.

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**REFERENCES**


### Table I

<table>
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<th>Protein</th>
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<td>2800</td>
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<tr>
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### Table II

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<td>1.3</td>
<td>0.023</td>
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<tr>
<td>Hb B</td>
<td>0.011</td>
<td>4.3</td>
<td>0.021</td>
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### Materials

- Hemoglobin, oxyhemoglobin, and deoxyhemoglobin (rabbit, human, and porcine) were obtained from Sigma Chemical Company, St. Louis, Missouri.
- Hb A and Hb B were purified by the method described by Wyman (1964).
- Hb A and Hb B were purified by the method described by Wyman (1964).

![Fig. 1A](image1.png) Hill plots of the oxygen equilibrium curves of Hb A, A, and Lepore Boston in 0.1 M phosphate buffer (pH 7.4) at 20°C.

![Fig. 1B](image2.png) Hill plots of the oxygen equilibrium curves of Hb A, A, and Lepore Boston in 0.1 M phosphate buffer (pH 7.4) at 20°C.

![Fig. 1C](image3.png) Hill plots of the oxygen equilibrium curves of Hb A, A, and Lepore Boston in 0.1 M phosphate buffer (pH 7.4) at 20°C.
Comparative studies of Hb Lepore Boston, Hb A2, and Hb A.
K Adachi, T Asakura, F M Gill and E Schwartz


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