OXYGEN EQUILIBRIUM OF HEMOGLOBIN HIROSHIMA

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Recently some abnormal hemoglobins with higher oxygen affinity were reported to occur in some cases with mild erythrocytosis (Charache et al., 1966; Nagel et al., 1967; Jones et al., 1967; Novy et al., 1967; Lines and McIntosh, 1967; Stamatoyannopoulos et al., 1968). Particular attention has been given to functional properties of these hemoglobins, because their structural alterations exist in the areas where the α- and β-chains may contact. These hemoglobins exhibited 4-5 fold increased oxygen affinity, apparently no heme-heme interactions ($n = 1$), but the normal Bohr effect. Shibata and Iuchi (1967) found a variant of hemoglobin, Hb Hiroshima, in individuals with erythrocytosis and revealed that histidine in position 143 of the β-chain was replaced by aspartic acid. In the present communication we will report that Hb Hiroshima shows unusual oxygen equilibrium behaviors characterized by 4-fold increased oxygen affinity, reduced heme-heme interaction ($n = 2.0$) and reduced Bohr effect (approximately two-thirds of the normal degree).

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Materials and Methods

Hemolysates from the Hb Hiroshima blood samples were freed from stroma and treated with the column of Sephadex G-25. Hb Hiroshima was isolated by a starch block electrophoresis in Tris-EDTA-borate buffer (pH 7.0) or by a column chromatography using Amberlite CG-50 equilibrated with 0.005 M ammonium phosphate buffer (pH 7.0). On chromatography the Hb Hiroshima fraction was readily eluted by washing the column with the equilibration buffer; the Hb A fraction remaining on the column was eluted with 0.5 M ammonium sulfate adjusted to pH 8.0. The abnormal component, Hb Hiroshima, consisted approximately 50% of the hemoglobin contents in the Hb Hiroshima blood. Oxygen equilibrium experiments were performed in potassium phosphate buffer at 20° and the oxygen dissociation curves were determined spectrophotometrically by the method described by Hayashi et al. (1966). The pH of the reaction mixture was determined with a glass electrode pH meter at the end of each experiment. Boyer's spectrophotometric method (Boyer, 1954) was employed for the titration of -SH groups in oxyhemoglobin with p-chloromercuribenzoate (PCMB).

Results

The oxygen dissociation curves of Hb Hiroshima and Hb A which were isolated by starch block electrophoresis, respectively, are shown in Fig. 1. The value for $P_{1/2}$ of Hb Hiroshima was about one-fourth that of Hb A. The values of n, which represent the interaction coefficient in Hill equation $Y = Kp^n/(1 + Kp^n)$, for Hb Hiroshima and Hb A were approximately 2.0 and 2.6, respectively, and as shown in Fig. 2, the n values for both hemoglobins

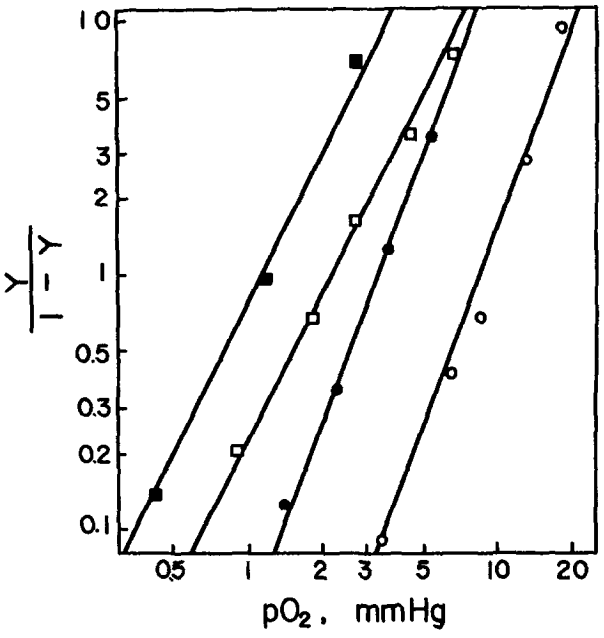

Fig. 1. Oxygen dissociation curves of Hb Hiroshima and Hb A. o——o, Hb A, pH 7.0; ●——●, Hb A, pH 7.8; □——□, Hb Hiroshima, pH 7.0; ■——■, Hb Hiroshima, pH 7.8. Hemoglobin, 5 x 10^{-5} M in heme. Buffers, 0.1 M phosphate. Lines in the figure represent the slope for $\underline{n}$ = 2.6 (Hb A) or $\underline{n}$ = 2.0 (Hb Hiroshima).

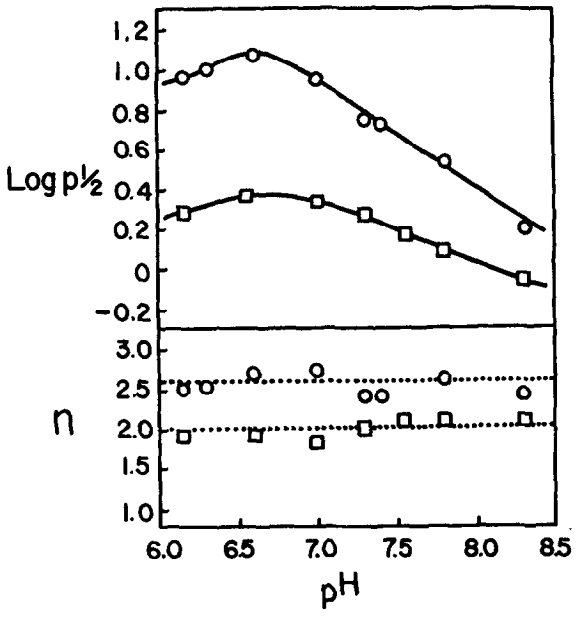

Fig. 2. Effect of pH on the oxygen equilibria of Hb Hiroshima and Hb A. o——o, Hb A; □——□, Hb Hiroshima. Conditions are similar to Fig. 1.

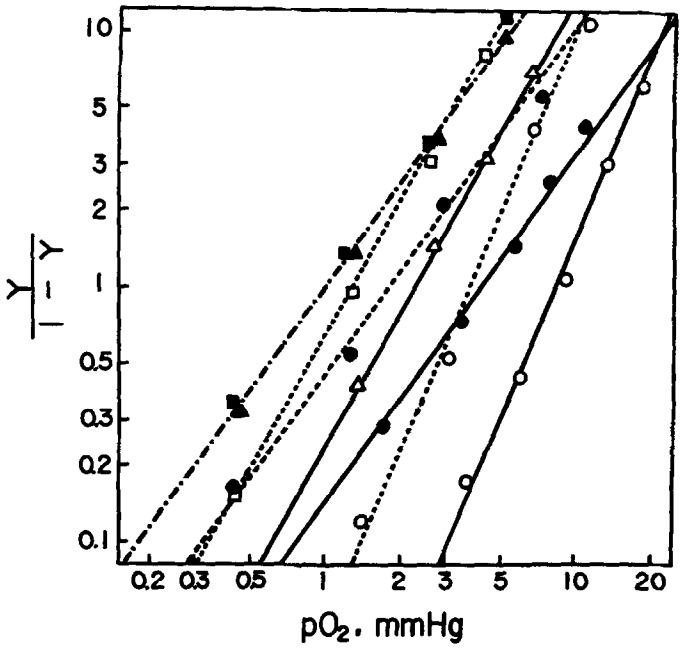

Fig. 3. Oxygen dissociation curves of Hb Hiroshima and Hb A in the presence (closed points) and absence (open points) of PCMB. o——o, ●——●, Hb A, pH 7.0; o·····o, ●·····●, Hb A, pH 7.8; Δ——Δ, ▲- - -▲, Hb Hiroshima, pH 7.0; □·····□, ■-·-■, Hb Hiroshima, pH 7.8. Hemoglobins, 5 x 10^{-5} M in heme. PCMB, 3 x 10^{-5} M. Buffers, 0.1 M phosphate. Lines in the figure represent the slope for n = 2.4 (o——o, o·····o), n = 1.8 (Δ——Δ, □·····□), or n = 1.4 (●——●, ●·····●, ▲- - -▲, ■-·-■).

were independent of pH ranging from 6.1 to 8.3. It is also evident in Fig. 2 that Hb Hiroshima has a considerably lower Bohr effect than Hb A. The value of $\Delta \log P_{1/2} / \Delta pH$ obtained in the pH range between 7.0 and 7.8 was 0.37 for Hb Hiroshima, while the value for Hb A was 0.57.

The titration of the -SH groups with PCMB demonstrated the existance of two reactive -SH groups per molecule of oxygenated Hb Hiroshima. This value is in agreement with that for Hb A (Benesch and Benesch, 1961; Snow, 1962). Oxygen dissociation curves of both Hb Hiroshima and Hb A fractions (isolated by Amberlite CG-50 column chromatography) determined in the pre-

sence of approximately 2.4 molecules of PCMB per molecule of hemoglobin are shown in Fig. 3. Apparently the n value of Hb Hiroshima was reduced from 1.8 to 1.4 by the -SH bloking with PCMB and also the value of $P^{1}/_{2}$ was lowered to about half that obtained without PCMB. These changes are similar to those observed for Hb A under the comparable experimental conditions. However, the Bohr effect of Hb Hiroshima disappeared when the -SH groups were blocked by PCMB, while the Bohr effect of Hb A was not appreciably affected by PCMB. The rate of autoxidation of Hb Hiroshima was lower (about two-thirds) than that of Hb A when tested at 37^{o} in 0.1 M acetate buffer, pH 5.0. Slower autoxidation of Hb Hiroshima may be related to its higher oxygen affinity.

Discussion

Studies on the relationships between structure and function of abnormal hemoglobins are valuable for the understanding of the mechanism of the conformational changes in hemoglobin molecule on oxygenation. Of a group of hemoglobin variants which were shown to have higher oxygen affinities (4 to 5-fold), markedly decreased n value (n = 1.0) and the normal Bohr effect, Hb Chesapeake (α92 Arg $\longrightarrow$ Leu; Charache et al., 1966), Hb J-Cape Town (α92 Arg $\longrightarrow$ Gln; Lines and McIntosh, 1967), and Hb Yakima (β99 Asp $\longrightarrow$ His; Jones et al., 1967) are characterized by the amino acid substitution in the FG or G region of the polypeptide chains. These regions are assumed to represent the areas of contact of α and β-chains (Perutz, 1965). Thus the functional abnormalities of these hemoglobin variants have been considered to reflect abnormal interactions between polypeptide chains. It is interesting in this connection that Hb Rainier (β145 Tyr $\longrightarrow$ His; Stamatoyannopoulos et al., 1968), having a substitu-

tion at residue H23 which is very close to the FG region of the same β-chain, showed the oxygen equilibrium behaviors very similar to those of Hb Chesapeake and Hb Yakima. In Hb Hiroshima the hisitidine H21 of β-chain is replaced by aspartic acid. According to the Perutz's model (Perutz, 1965) the position of H21 is close to the carboxyl-terminal region of the partner β-chain in hemoglobin molecule, but may not be directly related with the FG region. Still the functional properties of Hb Hiroshima were abnormal. Further, it was shown by Antonini et al. (1961) that when the carboxyl-terminal Tyr-His was removed from the β-chain by the carboxypeptidase A digestion, the resulting modified hemoglobin exhibited a very high oxygen affinity (30-fold), markedly decreased heme-heme interaction (n = 1.0) and practically no Bohr effect. All these findings point to the importance of amino acids in the carboxyl-terminal region of β-chain in the oxygenation of hemoglobin. The possibility may also be considered that the carboxyl-terminal region of β-chain plays a role in an integrated manner in the oxygenation reaction, although the degree of contribution of each amino acid with respect to the function of hemoglobin molecule may be different. In this respect, it is noteworthy that Hb Hiroshima still retained a considerable heme-heme interaction (n = 2.0) and the Bohr effect, though its degree was significantly reduced. The effects of alteration in the primary structure of Hb Hiroshima on the interaction between the hemoglobin chains seem to be rather moderate. The results obtained with PCMB may also support this view.

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