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THE OXYGEN EQUILIBRIA OF SOME "SLOW-MOVING" HUMAN HEMOGLOBIN TYPES

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SUMMARY

The equilibria between oxygen and four different kinds of abnormal hemoglobin, Hb-D_{Punjab}, Hb-D with another unknown abnormality in the β-chain, Hb-S and Hb-Lepore have been determined. Total red blood cell hemolysates as well as isolated components (DEAE-cellulose chromatography) were studied.

The two Hb-D abnormalities in isolated forms showed a slight but distinct increase in oxygen affinities, while their Bohr effects were not altered. Red blood cell hemolysates of sickle-cell anemia patients failed to show a significant change in the affinity for oxygen. The similarity of the oxygen equilibria of Hb-S and Hb-A containing hemolysates was demonstrated with the use of potassium phosphate buffer of varying molarities.

A marked increase in oxygen affinity of pure Hb-Lepore as compared to normal Hb-A was found. The equilibrium between oxygen and this Hb-type was comparable with those found for Hb-A_2 and its variant Hb-A_2'. No change in the Bohr effect was noted. A small but distinct increase in the oxygen affinity of a red blood cell hemolysate of a Hb-Lepore trait carrier was also demonstrable.

INTRODUCTION

With the discovery of numerous abnormal types of human hemoglobin it seems of importance to study possible alterations of one of the most important properties of such hemoglobin types, namely their affinity for molecular oxygen. It has already been demonstrated that certain alterations in the amino acid sequence of either the α- or the β-polypeptide chains will be reflected in an altered oxygen equilibrium. RIGGS AND WELLS[1], for instance, have presented data indicating a considerable decrease in the affinity for oxygen of sickle cell hemoglobin (designated as $\alpha_2\beta_2^{6val}$) as compared with normal Hb-A ($\alpha_2\beta_2$). Certain hemoglobin types, known as the abnormal Hb-M types, show a defect in their oxygen carrying capacity, since the iron atom of the heme group attached to the altered polypeptide chain is present in the ferric form instead of in the ferrous form. Examples of such abnormal types of methemoglobin are Hb-M_{Boston} ($\alpha_2^{58\,his}\beta_2$), Hb-$M_{Saskatoon}$ ($\alpha_2\beta_2^{\,63\,his}$), and Hb-$M_{Milwaukee\ I}$ ($\alpha_2\beta_2^{\,67\,glu}$) (see ref. 2). Differences in oxygen equilibrium have also been noted for hemoglobin types being composed of one type of polypeptide chain, such as

Hb-H (designated as β_4) and Hb-Bart's (designated as γ_4), or of α-polypeptide chains and δ polypeptide chains, such as the normally occurring minor hemoglobin component Hb-A_2 (designated as $\alpha_2\delta_2$) and its variant Hb-A_2' ($\alpha_2\delta_2{}^{A_2'}$). The oxygen affinities of both Hb-H (see ref. 3) and Hb-Bart's (see ref. 4) were found to be 10–12 times higher than that of normal Hb-A, while the oxygen affinities of Hb-A_2 and Hb-A_2' showed an increase of about 60 % (see ref. 5). Recently a possible abnormal form of adult hemoglobin has been described showing an extreme decrease in its affinity for oxygen[6].

In the present study we have determined the oxygen equilibria of two variants of the normal Hb-A, namely Hb-D_{Punjab} (see ref. 6) and a second different Hb-D type with an unknown abnormality located in the β-chain, and of Hb-Lepore[7–9], whose structural abnormality is still a subject of extensive studies[10,11]. In addition, we reinvestigated the oxygen affinity of sickle cell hemoglobin (Hb-S) because of the inconsistency of the results reported[1,12,13]. It will be noted that the four abnormal Hb-types show an almost identical decrease in their electrophoretic mobilities at alkaline pH.

EXPERIMENTAL

The Hb-D_{Punjab} was present in several heterozygous carriers of a family of apparently Caucasian origin. The identity of the Hb-type with Hb-D_{Punjab} was established by studying the tryptic digest of the pure hemoglobin variant with the use of the fingerprinting technique; an altered peptide, called number 5, was present in the β-chain of the molecule[14]. The second Hb-D type was found in a Negro, being also heterozygous for the hemoglobin abnormality. Structural studies failed so far to elucidate the chemical change in the molecule although its non-identity with Hb-D_{Punjab} could definitely be established. The abnormality was found to be located in the β-chain of the molecule. Two Hb-Lepore carriers were available; the family has been described before[9]. Several patients, homozygous for Hb-S, furnished blood samples for the study of the oxygen equilibrium of this abnormal hemoglobin type. Small quantities of fetal hemoglobin (Hb-F) were present in most of these samples, its percentage never exceeding the 10 %. Blood samples from normal individuals served as controls.

The red blood cells were washed 4 times with 0.9 % NaCl solution; the packed cells were lysed with an equal volume of distilled water and 0.4 volume of toluene. The hemolysates were cleared from debris by centrifugation. In most instances the hemolysates were diluted with distilled water until final hemoglobin concentration was 5 g% and dialyzed at 4° for 20 h against a large volume (at least 100 times that of the hemolysates) of potassium phosphate buffers at the specified molarities and pH values. The two Hb-D abnormalities and the Hb-Lepore were also isolated by DEAE-cellulose chromatography at 4° as described before[15]. Solutions of the abnormal Hb-types and the isolated normal Hb-A_0 were adjusted to a concentration of approx. 1 g %, dialyzed against distilled water at 4° for 4 h and *versus* potassium phosphate buffer at the specified molarities and pH values at 4° for 20 h. The purity of each isolated fraction was tested by starch-gel electrophoresis[16]. Only those hemoglobin samples were studied that did not contain any notable percentage (below 3 %) of ferrihemoglobin as demonstrated by spectral analyses.

The procedure for the determination of the oxygen affinities was a modification of that being described by BRINKMAN AND DIRKEN[17], which was also used in earlier studies[5,18,19]. The accuracy of the procedure was established by the simultaneous determination of the $\log P_{50}$ (*i.e.* oxygen pressure at 50% oxygenation) of eight identical solutions of normal Hb-A at different pH values; a standard deviation of the mean of 0.020 was found. All dissociation curves reported in this paper were made at 37°. At the end of each experiment, the pH of the hemoglobin solution was measured with a Radiometer 4 pH-meter. Since no difference in the Bohr effect was noted for the various Hb types, the values for the O_2 affinity were, when necessary, corrected to pH 7 (at 37°), taking into account the normal Bohr effect in this region of pH: $(d \log P_{50}/d\ pH)_T = -0.45$ (20).

RESULTS AND DISCUSSION

Hb-D$_{Punjab}$ and Hb-D$_{(\beta\text{-chain abn.})}$

Investigations of the oxygen affinities at different pH values of the red blood-cell hemolysates of the Hb-D trait carriers revealed no difference when compared with hemolysates of normal red blood cells. The shapes for the oxygen equilibrium curves, the Bohr effects and the P_{50} values at the different pH values were all identical. The factor n, defined as the slope of the line relating the $\log pO_2$ with $\log y/(1-y)$ (y = % oxygenation), was between 2.4 and 2.8. Small but consistent differences in the affinities for oxygen were noted, when the two isolated Hb-D types were compared with the isolated major normal Hb component. As demonstrated in Table I, the affinities for oxygen of Hb-D$_{Punjab}$ and of the second Hb-D abnormality were consistantly higher than that of Hb-A$_0$. This slight increase, being 2–3 times greater than the standard deviation of the mean of the determination, was found to be independent of the pH. Both isolated abnormal components therefore exerted the same Bohr effect. These results suggest that structural abnormalities of the two D hemoglobins, which are yet not definitely established, may interfere with the normal oxygen equilibrium resulting in a slight increase in their affinities for molecular oxygen. An alternate explanation may be that slight changes in tertiary structure have occurred during the isolation of the hemoglobin component. Although this second possibility cannot be excluded, it becomes less likely due to the results obtained for other Hb-types such

TABLE I

THE BOHR EFFECT OF THE ISOLATED Hb-A$_0$ AND TWO FORMS OF Hb-D (β-CHAIN ABNORMALITIES)

*pH** (at 37°)	*Hb-A$_0$* $\log P_{50}$	*Hb-D$_{Punjab}$* $-\Delta \log P_{50}$	*Hb-D$_{(\beta\text{-Chain})}$* $-\Delta \log P_{50}$
6.08	1.46	0.01	0.06
6.17	1.38	—	0.02
6.53	1.32	0.06	0.09
6.93	1.10	0.06	0.04
7.08	1.08	0.08	0.04
7.20	1.02	0.04	—
7.36	0.98	0.07	0.04
Mean		0.055	0.05

* Of isolated components dialyzed for 24 h against 0.1 M phosphate at 4°.

as Hb-Lepore (see below) and Hb-C. These two Hb types and the two Hb-D components behaved almost identically in DEAE-cellulose chromatography; nevertheless the increase in oxygen affinity of the isolated Hb-Lepore and the absence of any change in the oxygen affinity of the isolated Hb-C corresponded well with the results obtained in studying the hemolysates, in which these abnormal hemoglobins were present.

Hb-S

The results of studies of the oxygen equilibrium of Hb-S reported in the literature are controversial. BECKLAGE *et al.*[12] and RIGGS AND WELLS[1] have demonstrated a substantial increase in the oxygen pressure required for 50 % oxygenation of whole sickle-cell blood[12] or of red blood-cell hemolysates[1]. It has been suggested that this displacement of the oxygen dissociation curves of sickle cell blood to the right of the curves of normal blood is due to dialyzable factors[1,12]. The results obtained by RIGGS AND WELLS[1] in their study of the oxygen affinities of dialyzed hemolysates of red blood cells of sickle cell anemia patients has been explained by an indirect effect of the amino acid substitution on the oxygen equilibrium. These data are inconsistent with those of WYMAN AND ALLEN[13], who found no difference in the oxygen affinities of hemolysates of red blood cells of normal individuals and of sickle cell anemia patients. The experimental conditions, under which the two investigations were performed, were quite different. RIGGS AND WELLS[1] studied their hemolysates after dialysis against distilled water and after addition of potassium phosphate buffers to a final concentration of 0.1 M. The experiments by WYMAN AND ALLEN were performed on undialyzed hemolysates in 0.4 M phosphate solutions, while the presence of other hemoglobin components was not excluded. The influence of the salt concentration on the functional properties of human hemoglobin has recently been emphasized by ROSSI-FANELLI *et al.*[20]. These investigators demonstrated that both the shape of the oxygen dissociation curve and the oxygen affinity largely depend on the salt concentration in the medium, since increases in the N values and in the pO_2 pressures required for 50 % oxygenation were noted with an increase in the salt concentration.

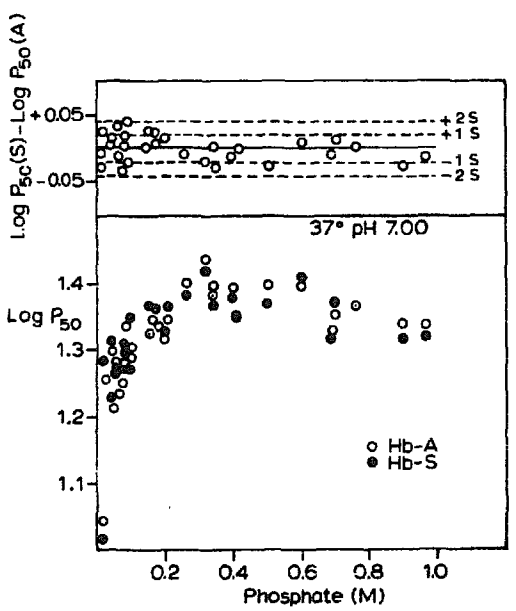

Fig. 1. The oxygen affinities of Hb-A and Hb-S containing red blood-cell hemolysates.

It seems, therefore, desirable to reinvestigate the oxygen equilibria of dialyzed hemolysates of normal red blood cells and of red blood cells of sickle cell anemia patients at different salt concentrations.

The results of such studies are presented in Fig. 1. Red blood-cell hemolysates of normal individuals and of sickle cell anemia patients, were dialyzed for 20 h at 4° against potassium phosphate buffers of molarities ranging from 0.01 M to 1.0 M. The oxygen dissociation curves of these solutions were determined in duplicate at 37°. The P_{50} values at a pH 7.00, calculated with the use of the formula mentioned earlier in this paper, were plotted against the determined phosphate concentrations of the hemolysates. A similar relation between the log P_{50} values and the phosphate concentrations as described by ROSSI-FANELLI *et al.*[20] was noted for both types of hemolysates. No significant differences were found between the P_{50} values and the shapes of the oxygen dissociation curves of the normal hemolysates and the Hb-S containing hemolysates at the several phosphate concentrations, including those used by RIGGS AND WELLS[1], namely 0.1 M, and by WYMAN AND ALLEN[13], namely 0.4 M. The values $\log P_{50}$ (S) $-\log P_{50}$ (A), *i.e.* the differences between the P_{50} values of the oxygen dissociation curves of the Hb-S and Hb-A containing hemolysates, were all smaller than two standard deviations of the mean. We, therefore, have to conclude that under our experimental conditions no significant decrease in the oxygen equilibrium of dialyzed red blood-cell hemolysates of sickle cell anemia patients could be demonstrated. It is noteworthy that the hemolysates used in this study contained mainly Hb-S, with 2–3 % Hb-A_2 and 0–10 % Hb-F; no significant amount of ferrihemoglobin was demonstrable.

Hb-Lepore

Examples of the oxygen dissociation curves of the red blood-cell hemolysates of a Hb-Lepore trait carrier containing approx. 10 % of the abnormal Hb, and of a normal individual are presented in Fig. 2A. A small increase in the affinity for oxygen can be noted. The shapes of the oxygen equilibrium curves, which were determined in an 0.1 M potassium phosphate buffer, were identical. A similar difference was seen at different pH values of the medium; the average decrease in oxygen pressure at 50 %

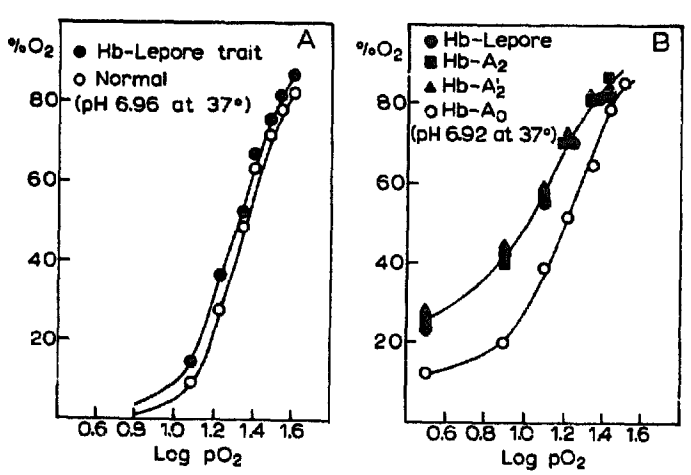

Fig. 2. The oxygen dissociation curves of Hb-Lepore. A, Total hemolysates of normal red blood cells and of erythrocytes of a Hb-Lepore trait carrier, containing 10 % Hb-Lepore; B, isolated Hb-components Hb-Lepore, Hb-A_2, Hb-A_2' and Hb-A_0.

oxygenation was found to be 6 %. The isolated Hb-Lepore showed a marked increase in oxygen affinity as compared with the isolated major normal Hb-component A_0; the average decrease in pO_2 necessary for 50 % oxygenation was approx. 40 %. The oxygen dissociation curve of Hb-Lepore was indistinguishable from those of the normal minor hemoglobin component A_2 and its variant Hb-A_2' (Fig. 2B and ref. 5). Due to the limited material that was available a definite opinion regarding the Bohr effect of pure Hb-Lepore could not be formed; the few oxygen equilibrium experiments carried out at different pH values in 0.1 M phosphate buffer suggested similar dependence on the pH of Hb-Lepore and normal Hb-A_0. The large increase in affinity for molecular oxygen of the isolated Hb-Lepore component corresponded quite well with the appreciable increase found for total red blood-cell hemolysates of Hb-Lepore carriers. The 4–5 % decrease in P_{50} value of such a hemolysate, which can be expected from the data obtained in studies of the isolated component, agrees with the decrease of 6 %, which was observed. The similarity of the oxygen equilibrium of pure Hb-Lepore and that of Hb-A_2 and Hb-A_2' suggests a close relation between these three Hb-types. Our data support the hypothesis that Hb-Lepore is a variant of Hb-A_2, being composed of normal α-chains and abnormal δ-chains[9,10], although structural studies have also indicated other possibilities[10,11].

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