BIPHASIC BLOOD OXYGEN DISSOCIATION CURVES IN HEMOGLOBIN S HEMOGLOBINOPATHIES. SICKLE CELL HETEROZYGOTES

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Summary: The oxygen binding curves of whole cells of heterozygous sickle cell hemoglobinopathies were measured. Under the conditions of measurement (T=25°, pH=6.4, pCO_2=0) the curves are biphasic. Samples in which 2,3-diphosphoglycerate (2,3-DPG) is very low or absent are monophasic, suggesting that there is a differential binding of 2,3-DPG to S and non-S hemoglobin in cells of heterozygotes. The biphasic curve has been confirmed in a hemoglobin SC patient, under physiologic conditions (T=37°, pH=7.4, pCO_2=44 mm).

It is now widely accepted that the oxygen dissociation curves of purified human hemoglobins A and S are essentially identical (1,2). However in hemoglobin S disease (sickle cell anemia) the oxygen dissociation curve is shifted to the right (3,4). It was suggested by Becklake et al., (3) that this right shift was due to dialyzable red cell factors, and the recognition of the importance of phosphates, especially 2,3-DPG, in hemoglobin oxygenation (5,6) indicated that the factor involved in sickle cell anemia impaired blood oxygenation might be 2,3-DPG. That this was indeed the case was shown by Charache et al., (2), who reported increased levels of erythrocyte 2,3-DPG in sickle cell anemia.

The situation with respect to hemoglobin S heterozygous conditions is less clear. Becklake et al., (3) found no differences between oxygen dissociation curves of normals and individuals with sickle cell trait (genotype AS, heterozygous for hemoglobin S), while Cawein et al. (7) reported a shift to the right of the oxygen dissociation curve in patients

with hemoglobin S-C disease (genotype SC, heterozygous for both hemoglobin S and hemoglobin C, no normal hemoglobin A). Levels of 2,3-DPG were not determined in these studies.

The present study was designed to evaluate blood oxygenation in hemoglobin S heterozygotes (A-S and S-C), and by comparison with blood-bank bloods, to evaluate the effect of 2,3-DPG depletion on oxygenation.

MATERIALS AND METHODS

Fresh blood samples from well adults heterozygous for hemoglobin S were obtained from the sickle cell anemia clinic and the Blood Bank of University Hospital, University of Ibadan (Nigeria). Expired blood-bank blood of A-S genotype was obtained from the University Hospital Blood Bank.

Oxygen dissociation curves were measured in a suspension of fresh erythrocytes by a modified polarographic method (8). The blood sample was introduced into a closed vessel, deoxygenated by beef heart particle succinate respiration (9), and pO_2 measured with a membrane type oxygen electrode (Radiometer E5040 with PHA927 gas monitor). The oxygen signal was continuously recorded on a Kipp and Zonen Micrograph BD5 recorder with automatic zero suppression. A complete description of the record and its analysis is given in the legend to Figure 1.

Controls run on each preparation of beef heart particles confirmed the linearity of deoxygenation in the absence of blood. Comparison of the method with a spectrophotometric determination of oxygen dissociation of purified human hemoglobin gave excellent agreement and validated the technique. A check of pH after the runs proved that no pH changes occurred.

RESULTS

The normal conditions for oxygen dissociation curves are pH≈7.4, pCO_2= 40 mm, T=37°. Because accurate gas mixtures containing CO_2 were unavailable, the experiments reported here were done on blood equilibrated with CO_2-free moist air. The reaction vessel was thermostated at 30°, and a pH (6.40) was chosen at which the $P_{1/2}$ of normal blood (hemoglobin A-A) was ca. 25 mm.

Under these conditions, the oxygen dissociation curve as measured on samples of either A-S or S-C blood was displaced to the right of the normal curve, although not to as great an extent as the dissociation curve of sickle cell anemia blood.

However, the most striking feature of the traces from hemoglobin S heterozygotes is that the curve was biphasic. Figure 1 shows one case of hemoglobin S-C, in which the "notch" separating the two parts of the dissociation curve is readily observed. This is a selected sample; the more common type of curve is one in which the two portions are incompletely resolved, with a linear region between the initial and final downward curvature.

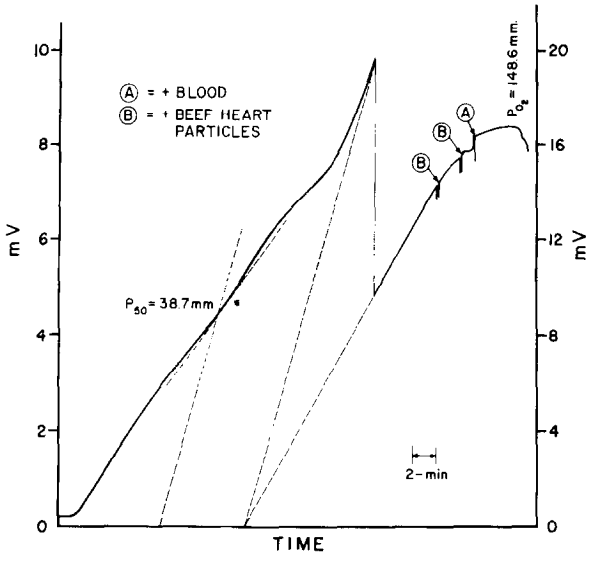

Figure 1. Oxygen dissociation curve of an erythrocyte suspension of fresh blood from a patient with hemoglobin S-C. The trace extends from right to left on the figure; the left half of the trace, which covers the entire dissociation curve, is on an expanded scale (0-10 mV, left ordinate). The buffer, 18 ml. of 0.25 M PO_4, pH=6.40, T=30°, with 0.007 M Na_2-succinate as substrate for respiration, in a closed vessel, was equilibrated with CO_2-free air (pO_2=148.6 mm). At A 0.5 ml of fully oxygenated blood was introduced; at B ca. 0.05 ml. of a suspension of beef heart particles. The initial straight line portion of the curve is the linear respiration of the particles, and is extrapolated to pO_2=0. The deviation of the trace from the control represents the oxygen dissociation curve of the sample; the ordinate (output of the oxygen electrode) is proportional to pO_2, and the abscissa, since metabolic deoxygenation is linear, is proportional to % saturation of the hemoglobin. A line parallel to the calibration line halfway between the extrapolated and experimental zero cuts the trace at an oxygen pressure equal to the half-saturation pressure (normal for hemoglobin A-A bloods, ~ 25 mm). A tangent to the trace has been drawn at the point (*) to emphasize the "notch" in the biphasic curve.

Recent studies of a case of hemoglobin S-C disease at the University of Miami School of Medicine have confirmed the biphasic oxygen dissociation curve at pH 7.41, 37°, pCO_2=44 mm.

This biphasic character of the dissociation curve was invariably present in fresh samples of S heterozygotes, whether A-S or S-C. In no case was the normal smooth sigmoid dissociation curve of normal blood noted. Blood samples from cases of sickle cell anemia, while displaced to the right as expected (2), were invariably monophasic sigmoid curves.

The result cannot be due to a dual cell population (S and non-S cells) since at pO_2 = 0 all cells in A-S or S-C heterozygotes sickle, and hence all cells contain hemoglobin S.

It is suggested that the biphasic curve is due to a differential interaction of 2,3-DPG with S and non-S hemoglobin in the erythrocyte. This hypothesis is supported by experiments on samples of expired blood bank A-S blood, in which the 2,3-DPG has been depleted. The oxygen dissociation curve was shifted back to the left (as expected when 2,3-DPG is depleted), and was monophasic.

DISCUSSION

Such a biphasic oxygen dissociation curve has not previously been reported in heterozygotes, possibly because only a few points on the curve were obtained and the deviation from a smooth monophasic curve was not apparent. In a reported study of a thalassemia neonatal blood sample, (10), the dissociation curve can however be drawn as a biphasic curve. Such a phenomenon might be expected for bloods with high proportions of hemoglobin F, since it has been shown that the response of hemoglobin F to 2,3-DPG is significantly reduced as compared to hemoglobin A (11).

The hypothesis that the biphasic curve is due to differential interaction of hemoglobin S and hemoglobin A or C with 2,3-DPG is contrary to the findings of Bunn *et al.*, (11), who found an approximately equal effect of 2,3-DPG in decreasing oxygen affinity. The right shift of the oxygen dissociation curve

on adding 2,3-DPG to hemoglobin S was greater than that for hemoglobin A or C, but the difference was not significant. A differential interaction sufficient to yield the biphasic curve shown in Figure 1 is within the reported experimental error (11).

The hypothesis is quite consistent with the findings of Thompson et al., (12), who found that the oxygen dissociation curves of hemoglobin S and A were identical at low (0.05 M) and high (> 0.4 M) concentrations of phosphate buffer, while at intermediate concentrations the curve for hemoglobin S was to the right of that for hemoglobin A. This might also explain the discrepencies in early comparative studies of oxygen dissociation curves of hemoglobins S and A; Riggs and Wells (13) found a significant difference in 0.1 M PO_4 while Wyman and Allen (14) found no difference in 0.4 M PO_4.

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