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HEMOGLOBIN SOLUBILITY AS A FUNCTION OF FRACTIONAL OXYGEN SATURATION FOR HEMOGLOBINS IN POLYETHYLENE GLYCOL: A SICKLE HEMOGLOBIN MODEL

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SUMMARY

The solubilities of hemoglobins S, A, and C in aqueous polyethylene glycol (M_n=6000) solutions were determined at fractional oxygen saturation levels from zero to one. A theory, based on the MWC two state model is presented which accurately describes hemoglobin solubility at any fractional saturation based only on knowledge of soluble hemoglobin oxygen binding and endpoint solubilities. Evidence of a predicted triple point is clearly seen for hemoglobin C.

INTRODUCTION

Hofrichter (1) and others (2,3) have studied the solubility of HbS gel as a function of fractional saturation. Such studies are pertinent because HbS containing red cells are rarely in the fully deoxygenated state in the peripheral circulation where the sickling process is thought to be most significant (5).

Ross et al. (6) and Minton (4) view the gelation as a two state phase transition in which HbS solution phase "monomers" are in equilibrium with gel phase "polymers" in a HbS gel. The solubility can be predicted from linkage theory (7) and the phenomena can be described generally by the concept of "polyphasic linkage" discussed by Gill and Wyman (8). We have recently shown the experimental validity of some of the predictions made by the polyphasic linkage model (9). We have examined the solubility behavior

Abbreviations used are: Hb, hemoglobin; HbA, normal adult hemoglobin; HbS, β6 Glu-Val variant; HbC, β6 Glu-Lys variant; PEG polyethylene glycol (M_n = 6000).

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of HbA, HbS, and HbC at fractional oxygen saturation from $\bar{Y} = 0 \rightarrow 1$ and propose a simple model to explain this data.

MATERIALS AND METHODS

HbA from outdated blood bank blood and HbS from homozygous volunteers were purified as described by Huisman and Dozy (10). HbC was purified from AC blood by chromatography on Sephadex CM 52 (11). PEG was obtained from Eastman Chemical Co.

Determination of solubility as a function of pO_2 was made at 17.5% PEG for HbA and HbS with total hemoglobin concentration of 0.08 mM Hb (tetramer) in 0.1 M potassium phosphate, pH 7.02 at 25 ± 1°C. For HbC the PEG concentration was reduced to 15% to produce workable solubilities. Other conditions were identical for HbA, HbS and HbC.

Solubility experiments were carried out in a 1 cm pathlength pyrex cuvette made with a screw top fitting for a cap with a rubber septum (from Technical Consulting Service Co., Southampton, PA). Gas equilibration, centrifugation, and spectrophotometry were done in the same cuvette. Fully deoxy- and oxyhemoglobin samples were equilibrated with pure N_2 and O_2, respectively. Other oxygen partial pressures were produced electrolytically in a purified N_2 stream. The gas was gently bubbled through the sample in the capped cuvette until precipitation and oxygen binding reached steady values. Surface denaturation due to bubbling was not noted.

Each Hb-PEG sample was prepared immediately prior to gas equilibration since time dependent oxidation of Hb due to the PEG or an impurity in it was noted. Methemoglobin as well as oxy- and deoxyhemoglobin concentration was determined spectrophotometrically. Since the concentration of methemoglobin present was essentially constant for all fractional saturations for each Hb the fraction of methemoglobin present at low pO_2 (and hence at low solubility) was relatively higher than present at higher pO_2. The increase in methemoglobin containing hybrids had little effect on solubility or soluble phase oxygen binding. For all samples, after gas equilibration, the capped cuvettes were centrifuged at 25°C for 5 min at 5000 xg prior to determining the solubility and the oxy-, met- and deoxyhemoglobin composition of the supernatant.

RESULTS

Oxygen binding data for soluble HbA in the presence and absence of 17.5% PEG are shown in Fig. 1. The difference between the two curves is small. Oxygen binding for soluble HbS and HbC are identical to that for HbA under the same conditions. The logarithm of the solubility (mM_{tet}) as a function of liquid phase fractional oxygen saturation is shown in Fig. 2 for HbA, HbS and HbC.

THEORY

In order to describe the solubility in relation to oxygen binding we make use of the polyphasic linkage theory of Gill and Wyman (8). Under

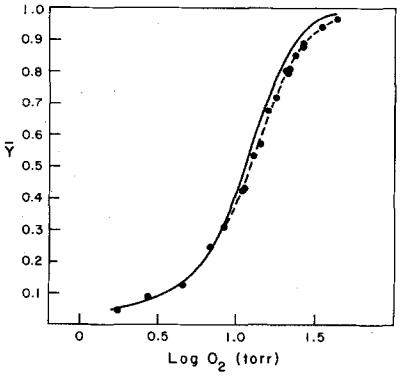

Fig. 1. Oxygen binding of HbA with (---) and without (——) 17.5% PEG. The dashed curve is fitted to the oxygen binding data in the presence of 17.5% PEG using MWC constants as follows: $L = 3 \times 10^{10}$, $k_T = 0.026$ torr^{-1} and $k_R = 39$ torr^{-1}. The data points represent the behavior in the presence of 17.5% PEG.

conditions of ideal behavior (i.e. low solubility) Wyman (7) has expressed the dependence of Hb solubility, c, (here equal to activity) on oxygen pressure, x, as:

$$\frac{d\ln c}{d\ln x} = \bar{X}^L - \bar{X}^S \qquad (1)$$

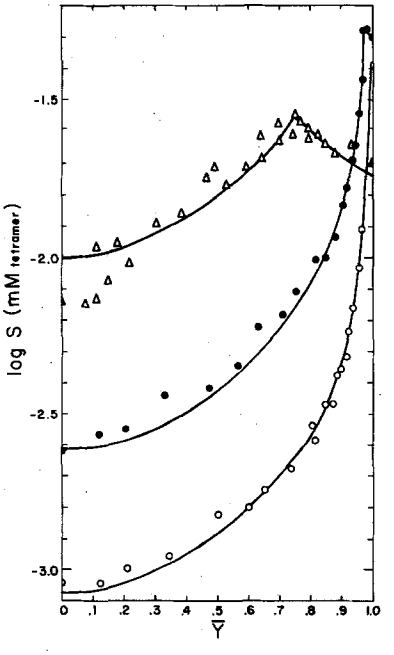

Fig. 2. Solubility of HbA ● , HbC △ and HbS ○ as a function of liquid phase fractional oxygen saturation. Curves are calculated from equations (3) and (4) using MWC constants from Fig. 1 and endpoint solubilities indicated on this figure.

where $\bar{X}^L$ and $\bar{X}^S$ are the amounts of oxygen bound per mole of hemoglobin in the liquid and solid phases, respectively. Integration from c_o, the solubility of pure deoxyhemoglobin, to any solubility, c, gives the desired relation with oxygen binding in the two phases. This is more readily seen in terms of the binding partition functions for oxygen binding in the liquid and solid phase, Q^L and Q_o^S, respectively. The relation of $\bar{X}^L$ and Q^L is determined by $\bar{X}^L = (d\ln Q^L)/(d\ln x)$. The logarithm of the binding partition function, Q^L, is given by the binding curve integral

$$\ln Q^L = \int_{-\infty}^{\ln x} \bar{X}^L \, d\ln x \qquad (2)$$

with an analogous relationship for the solid phase term, Q_o^S. The overall result is simply

$$\frac{c}{c_o} = \frac{Q^L}{Q_o^S} \qquad (3)$$

Equation (1) can also be integrated from high oxygen pressures where all of the Hb in both phases is fully oxygenated. By analogy to the development of equation (3), integration from the oxyhemoglobin side yields:

$$\frac{c}{c_\infty} = \frac{Q^L/\beta_4^L}{Q_\infty^S/\beta_4^S} \qquad (4)$$

where c_∞ is the solubility of the fully oxygenated Hb, β_4^L is the overall binding constant for four oxygen molecules to Hb in solution, Q_∞^S is the binding partition function to the solid formed at high oxygen pressure and β_4^S is the overall binding constant of four oxygens in this solid phase.

We now use the results of the MWC model (12) to describe the binding partition functions in equations (3) and (4):

$$Q^L = \frac{L}{1+L}(1 + k_T x)^4 + \frac{1}{1+L}(1 + k_R x)^4 \qquad (5)$$

$$Q_o^S = (1 + k_T x)^4 \qquad (6)$$

$$Q_\infty^S = (1 + k_R x)^4 \qquad (7)$$

$$\beta_4^S = (k_R)^4 \tag{8}$$

$$\beta_4^L = (Lk_T^4 + k_R^4)/\ (1 + L) \tag{9}$$

where L, k_T and k_R are defined by the liquid phase binding curve. Equation (6) expresses the assumption that Q_o^S is determined by binding to a single allosteric form, namely, the T form, with a binding constant k_T, which is the same as that given in the liquid phase. Likewise, equation (7) determines Q_∞^S as the effect of binding to the single allosteric R form with the binding constant k_R. The overall binding constant to the R form in the solid state is expressed by equation (8). Equation (9) gives the overall binding constant for the liquid phase.

The condition of oxygen pressure where the two different solid state forms (T and R) coexist defines a triple point. At oxygen partial pressures below this point, we have assumed the T form is the stable state of the solid, and above this point, the R form is the stable state of the solid.

The MWC constants derived from the data in Fig. 1 enable us to use equations (3) and (4) along with the deoxy and oxyhemoglobin solubilities to predict the solubilities as a function of the fractional degree of oxygen saturation in the liquid phase. The results are shown by the solid curves in Fig. 2 for HbA, HbS, and HbC.

DISCUSSION

As seen in Fig. 2 the line for the theoretically generated solubilities agrees very well with the experimental determinations. The striking feature of these results is that the solubility of all three hemoglobins, HbA, HbS, and HbC, can be predicted by the same binding constants, which are derived from the liquid phase data. The satisfactory application of the affinities k_T and k_R to the solid phases suggests that binding in the solid phase is similar to the binding by the T and R liquid forms. Presumably, the crystal lattice does not effect the affinity of a particular form, but serves to maintain either the R or T forms.

The point at which both R and T crystal forms can coexist defines the triple point. The structural uniqueness of the two forms is supported by observations of Haurowitz (13) and Perutz (14) who showed that deoxyhemoglobin crystals shatter upon oxygenation. The simple theory we have employed to predict solubility behavior with oxygenation shows that the triple point of HbA and HbS will occur at extremely high degrees of liquid phase saturation due to the much larger solubility of the R or oxyhemoglobin form. Thus it is difficult to experimentally define this point from solubility data alone. However, for the case of HbC where the solubility of oxy and deoxy forms are comparable, the triple point can be observed at a moderate degree of saturation. This point is clearly defined in Fig. 2.

In order to further examine the binding features of these systems direct measurements on the oxygen binding in the solid phase are being pursued.

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