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THE EFFECT OF NON-BINDING MOLECULES ON THE GELATION OF HbS

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The influence of an inert globular macromolecule upon the solubility of sickle cell hemoglobin has been determined as a function of the degree of oxygenation. The thermodynamic theory required to treat this and related problems is derived starting with the Gibbs-Duhem equation and introducing the effect of specific binding (oxygen) by use of the binding partition function. The treatment includes non-ideal solution behaviour as measured by osmotic pressure of highly concentrated macromolecular solutions. Application of the theoretical equations demonstrates how the solubility of hemoglobin is influenced by the presence of the binding ligand (oxygen) and the inert macromolecule, bovine serum albumin (BSA). Good agreement is obtained between experimentally determined and theoretically calculated solubilities using 1) oxygen binding curves to solution and gel phases, 2) activity coefficients from osmotic pressure data, 3) one solubility under the condition where oxygen and BSA are absent, and 4) the value of the water content of the gel phase. Examination of the theoretical equations suggests that inert molecules of intermediate size, that are partially excluded from crystalline or gel phases, have the potential of generally increasing the solubility when non-ideal solution effects are small.

1. Introduction

In previous studies [1,2] of sickle cell hemoglobin gelation we have shown how oxygen binding curve information can be used in conjunction with solubility studies to give a comprehensive thermodynamic picture of this important oxygen-linked phase separation process [3]. Our current aim is to extend these results to situations where non-binding, generally large, molecules are added to the solution of sickle cell hemoglobin. The first goal is to consider the general thermodynamic formulation of the effect such molecules have on the non-ideal properties of concentrated solutions and the effect on the solubility of sickle cell hemoglobin when these molecules are excluded from the gel phase. This involves a thermodynamic analysis of macromolecular two phase equilibria. Minton [4–7] has considered the same general phenomena from the viewpoint of statistical thermodynamics in which various models form the basis of comparison with experimental results. In contrast we have chosen to develop the description of ligand linked phase phenomena upon purely thermodynamic grounds where the descriptive parameters of the system are accessible by experimental measurements. The two approaches ultimately complement each other in giving an in depth view of the role various ligands play upon phase separation phenomena as found in the sickle cell hemoglobin systems.

A second goal is to apply these formulations to examine the specific effect of added serum albumin upon the solubility of sickle cell hemoglobin in various states of oxygenation. Ross and Minton [8] have shown how osmotic pressure data on concentrated hemoglobin solutions [9] can be analyzed by a simple hard sphere model and have included the effect of additional spherical molecules upon the non-ideal solution properties of mixtures [10]. Their results provide a practical basis for calculation, by purely thermody-

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namic means, of the solubility of sickle cell hemoglobin under various conditions of oxygen partial pressure and added non-binding molecules once the basic equations describing the phase equilibria are available.

Although the application of the theoretical analysis of the influence of non-binding molecules upon the solubility of macromolecules is motivated towards the case of sickle cell hemoglobin, the general analysis of this phenomena should have a broad range of application to multiple phase macromolecular systems.

2. Experimental

The oxygen binding curves on concentrated hemoglobin solutions were obtained by use of the thin film method [1,11]. The detection of phase separation and the measurement of the extent of phase separation was accomplished by determinations of birefringence as described by Gill et al. [2]. These measurements enable the solubility to be determined as a function of oxygen partial pressure under conditions of varying amounts of added serum albumin.

Sickle cell hemoglobin was prepared from homozygous sources by removal of diphosphoglycerate and concentrated to approximately 0.4 g/ml. Less than 1% of the total hemoglobin was judged to be fetal hemoglobin. Pellets of the HbS were stored under liquid nitrogen. Examination of the thin layer spectra of samples studied indicated that methemoglobin formation was less than five percent. Concentrations of HbS samples were determined by duplicate determinations according to the Drabkin procedure [12]. This method gave results in agreement with direct determinations of the optical absorption of the undiluted oxygenated sample placed in a 0.100 mm optical cell and using an optical absorption coefficient of 14.6 mM^{-1} at 577 nm [13]. All solutions were buffered in 0.15 M phosphate, which was chosen as the standard buffer solution [14]. Solutions with various amounts of serum albumin were prepared by adding serum albumin to a standard sickle cell hemoglobin solution such that the final hemoglobin concentration was 0.243 g/ml.

3. Results

The influence of serum albumin at high concentration (0.20 g/ml) upon the oxygen binding curve under non-gelling concentrations of hemoglobin was found to be negligible (see fig. 1). The effect of serum albumin upon the solubility of HbS as a function of oxygen partial pressure is shown in fig. 2. The results shown in this figure were obtained from observations of the birefringence that occurs once gelation takes place at specific oxygen partial pressures. The amount of gel formed is estimated from the birefringence as shown by Gill et al. [2]. Since the serum albumin is excluded from the gel [15] its liquid phase concentration changes along these experimental solubility curves. One calculates this concentration from simple mass conservation requirements along with the hemoglobin concentration of the gel phase. The pertinent ideas are as follows: The concentration of serum albumin in the liquid phase, c_A^L, is equal to $c_A^T/(1-\Phi)$ where c_A^T is the total concentration of BSA in the original, non-gelled solution and Φ is the volume fraction of the gel phase. The volume fraction Φ is given [6] by $(c_M^T - c_M^L)/(c_M^S - c_M^L)$ where c_M^T is the total hemoglobin concentration in the original, non-gelled solution, c_M^L is the hemoglobin concentration in the liquid phase, and c_M^S is the hemoglobin concentration in the gel [6]. The weight

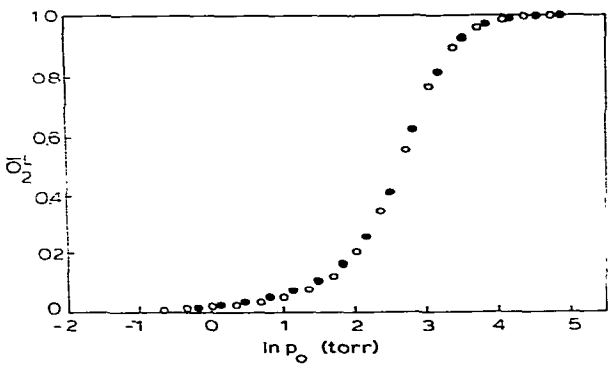

Fig. 1. Oxygen binding curves of sickle cell hemoglobin under non-gelling conditions in the presence and absence of bovine serum albumin (BSA). Experimental conditions are 0.075 g/ml HbS at pH 6.90 and 25°C. Control run without BSA denoted by •. Comparative run with BSA (0.20 g/ml) denoted by ○. Adair constants for control run data are $K_1 = 0.105$ $Torr^{-1}$, $K_2 = 0.026$ $Torr^{-1}$, $K_3 = 0.0144$ $Torr^{-1}$, and $K_4 = 1.08$ $Torr^{-1}$.

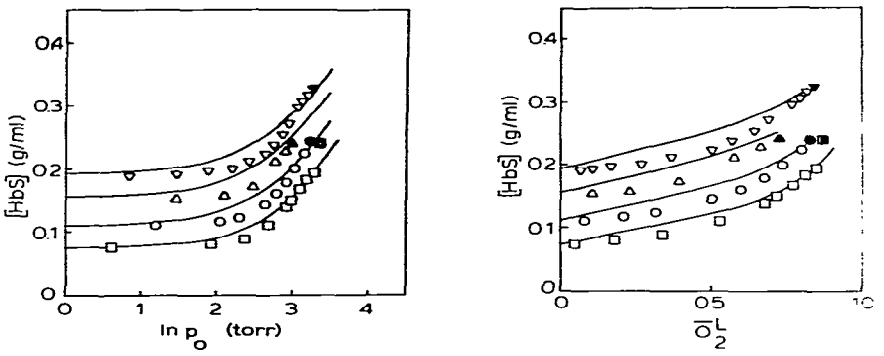

Fig. 2. The effect of BSA upon the solubility of HbS as a function of oxygen partial pressure (p_O), left, and extent of oxygen binding to the liquid phase ($\bar{O}^L = \bar{X}^L$), right. Data points were taken from birefringence measurements converted to solubility by scaling to deoxygenated hemoglobin solubility of Behe and Englander [16]. See text for details. Solution conditions similar to those of fig. 1. Initial concentration conditions are denoted as follows: HbS (0.332 g/ml), BSA (0.0 g/ml), ▽; HbS (0.243 g/ml), BSA (0.047 g/ml), △; HbS (0.243 g/ml), BSA (0.098 g/ml), ○; HbS (0.243 g/ml), BSA (0.141 g/ml), □. The disappearance point of birefringence is denoted by filled symbols for the appropriate initial solution conditions. Solid lines are the result of theoretical calculations described in text, using Adair constants of fig. 1 for liquid phase, $\bar{K}^S = 0.01$ Torr^{-1} for the solid phase, $\bar{c}_M^{OL} = 0.19$ g/ml, and $\bar{c}_M^S = 0.7$ g/ml.

fraction of the total protein that is gel, f, is simply related to the volume fraction by $f = \Phi c_M^S/(c_M^T + c_A^T)$. The weight fraction f is proportional to the birefringence. The proportionality factor is determined from the amount of birefringence in the fully deoxygenated state where the solubility of hemoglobin in the presence of BSA is known. This solubility data is given by the work of Behe and Englander [16]. Values of c_M^L can then be calculated from the amount of birefringence for a given experiment according to

$$c_M^L = \frac{c_M^T - f(c_M^T + c_A^T)}{1 - f(c_M^T + c_A^T)/c_M^S} . \quad (1)$$

The value of c_M^S has been determined to be 0.7 g/ml by Sunshine et al. [17].

4. Theory

In this section we develop the necessary thermodynamic equations for expressing the effect of ligand binding, and the effect of an inert non-binding molecule upon the solubility of sickle-cell hemoglobin under highly non-ideal solution conditions. The system can be regarded to be made up of the following components: unligated hemoglobin (M), solvent water (W), oxygen (X), other ligand (Y), and non-binding added molecule (A). The temperature and pressure are regarded as fixed. The Gibbs-Duhem equation for a phase denoted by superscript α (L for liquid and S for gel solid) is

$$n_W^\alpha \, d\mu_W + n_X^\alpha \, d\mu_X + n_Y^\alpha \, d\mu_Y + n_A^\alpha \, d\mu_A + n_M^\alpha \, d\mu_M = 0 . \quad (2)$$

Here the moles of the various components in the given phase are denoted by n_i^α where the subscript i stands for one of the five components. The chemical potentials of the respective components are denoted by μ_i. Since its value is the same in both phases in equilibrium with each other no superscript designation is needed.

With a two phase system there are two equations of the form of eq. (2) and thus one of the chemical potential terms may be eliminated. To do this we normalize one of the components in each phase. For convenience we choose to normalize to the water component in each phase. The result for the differential of the chemical potential for water in the solid phase is

$$d\mu_W = \frac{n_M^S}{n_W^S} \, d\mu_M + \frac{n_X^S}{n_W^S} \, d\mu_X + \frac{n_Y^S}{n_W^S} \, d\mu_Y + \frac{n_A^S}{n_W^S} \, d\mu_A \quad (3)$$

and in the liquid phase is

$$d\mu_W = \frac{n_M^L}{n_W^L} d\mu_M + \frac{n_X^L}{n_W^L} d\mu_X + \frac{n_Y^L}{n_W^L} d\mu_Y + \frac{n_A^L}{n_W^L} d\mu_A . \quad (4)$$

For practical reasons we usually wish to refer to moles of the various components per mole of hemoglobin. For example the moles of water per mole hemoglobin in the solid might be designated by $n_W^S/n_M^S = \bar{W}^S$. In general we shall use the bar to refer to moles of hemoglobin. Thus the moles of any component C per mole of hemoglobin in phase α is denoted as follows:

$$\bar{C}^\alpha = n_C^\alpha / n_M^\alpha . \quad (5)$$

With this notation we may write the combination of eqs. (3) and (4) as follows:

$$\left(\frac{1}{\bar{W}^S} - \frac{1}{\bar{W}^L}\right) d\mu_M + \left(\frac{\bar{X}^S}{\bar{W}^S} - \frac{\bar{X}^L}{\bar{W}^L}\right) d\mu_X + \left(\frac{\bar{Y}^S}{\bar{W}^S} - \frac{\bar{Y}^L}{\bar{W}^L}\right) d\mu_Y + \left(\frac{\bar{A}^S}{\bar{W}^S} - \frac{\bar{A}^L}{\bar{W}^L}\right) d\mu_A = 0 . \quad (6)$$

In principle all the terms in parentheses in eq. (6) are measurable by direct chemical analysis. The measurement of the chemical potentials depends upon separate experiments such as the determination of partial pressures for gases or osmotic pressures for macromolecules. The chemical potential is conveniently expressed by the activity for the ith component, a_i, according to $\mu_i = \mu_i^0 + RT \ln a_i$. In the case of the oxygen component, the activity would be given by either the oxygen partial pressure, p_X, or by the free and unligated oxygen concentration irrespective of the amount of oxygen that is bound to the hemoglobin macromolecule. The activity of the hemoglobin component M likewise depends upon the amount of hemoglobin that is unligated. We shall designate the fraction of unligated hemoglobin by α_0. The measurement of osmotic pressure determines the total activity of all non-diffusible macromolecules, here hemoglobin, in whatever state of ligation. We shall denote the total or osmotic activity of all hemoglobin by a_T. The suitable activity of the unligated macromolecular species, a_M, may be expressed as the portion of the total macromolecular activity contributed by the unligated species and may be written as follows:

$$a_M = \alpha_0 a_T , \quad (7)$$

For the case of oxygen binding to hemoglobin the activity coefficients of all intermediate species of oxygenated hemoglobin are apparently equal [7].

The chemical potential for the unligated species is given as

$$\mu_M = \mu_M^0 + RT \ln a_M ,$$

$$\mu_M = \mu_M^0 + RT \ln \alpha_0 + RT \ln a_T , \quad (8)$$

where R is the gas constant, T the absolute temperature, and μ_M^0 the standard state value of the chemical potential. In writing this result we have made use of eq. (7).

The fraction of unligated macromolecules α_0 is directly related to the binding polynomial or partition function denoted by Q [18–20] as follows:

$$\alpha_0 = 1/Q . \quad (9)$$

The binding polynomial expresses the information about the oxygen and Y binding reactions within the system. The overall binding reactions are specified as follows:

$$M + iO_2 + jY \rightarrow M(O_2)_i Y_j \quad (10)$$

where i ranges from 0 to 4 for hemoglobin and where j ranges over the number of Y ligands bound. The equilibrium constant for each reaction (10) is denoted by β_{ij}. The binding polynomial Q is given in terms of these binding reactions as

$$Q = \sum\sum \beta_{ij} p_X^i a_Y^j , \quad (11)$$

where p_X and a_Y are the oxygen pressure and Y ligand activities. An important property of the partition function is that it gives the amount of bound ligand per mole of macromolecule in the case of either the oxygen or Y component, as the logarithmic partial derivative of Q with respect to the logarithmic activity of that component. This result is expressed as follows:

$$\bar{C}_B^\alpha = \frac{\partial \ln Q}{\partial \ln a_C} , \quad (12)$$

where $\bar{C}_B^\alpha$ is the moles of component C bound per mole of macromolecule in phase α, and a_C is the activity of that component.

We now proceed to calculate the differential of μ_M in these terms for the liquid phase. First we introduce eq. (9) into (8) and differentiate in terms of the

oxygen activity p_X and the Y ligand activity a_Y. Then we identify the partial derivatives in terms of bound X and Y by eq. (12). The result is

$$d\mu_M = RT(-\bar{X}_B^L \, d\ln p_X - \bar{Y}_B^L \, d\ln a_Y + d\ln a_T) . \quad (13)$$

This gives the important operational result of the way in which the chemical potential of the unligated macromolecule is affected by the extent of ligation by oxygen and Y and the osmotic activity (a_T) of the hemoglobin solution.

The total amount of oxygen or Y per mole of hemoglobin is the sum of the free and the bound. In phase α we let $\bar{C}^\alpha$ represent the sum of the amount of component C that is free ($\bar{C}_F^\alpha$) plus the amount bound ($\bar{C}_B^\alpha$);

$$\bar{C}^\alpha = \bar{C}_F^\alpha + \bar{C}_B^\alpha . \quad (14)$$

With these results we may combine eq. (13) with eq. (6). We make use of appropriate activity expressions for the chemical potential of the various components and the expressions given by eq. (14) for the free and bound components. The final result is

$$d\ln a_T = \frac{(\bar{X}_B^L - \bar{X}_B^S) + \bar{W}^S[\bar{X}_F^L/\bar{W}^L - \bar{X}_F^S/\bar{W}^S]}{1 - \bar{W}^S/\bar{W}^L} d\ln p_X$$

$$+ \frac{(\bar{Y}_B^L - \bar{Y}_B^S) + \bar{W}^S[\bar{Y}_F^L/\bar{W}^L - \bar{Y}_F^S/\bar{W}^S]}{1 - \bar{W}^S/\bar{W}^L} d\ln a_Y$$

$$+ \frac{\bar{W}^S[\bar{A}^L/\bar{W}^L - \bar{A}^S/\bar{W}^S]}{1 - \bar{W}^S/\bar{W}^L} d\ln a_A . \quad (15)$$

Only the total amount of water per mole of hemoglobin in each phase, solid and liquid, has been specified. A division into bound and free water has been discussed by Tanford [21], but the necessary information to make this separation is usually not available.

We note that eq. (15) reduces to a result (2) derived for the linkage of oxygen binding to the solubility of sickle cell hemoglobin, provided the free oxygen and Y are equally distributed in the solvent W of the two phases and component A is absent. In such cases, the first and second terms in brackets [ ] will be zero.

We have assumed that there is no binding of the serum albumin component A to hemoglobin. Thus the third bracketed term in eq. (15) represents only unbound albumin. Again we note that if that component partitions equally between the water in each phase then it will have no effect on the hemoglobin solubility. However, we are primarily interested in the situation where the added inert macromolecule A is excluded from the solid phase. The bracketed term is therefore important in this situation.

For this special case which applies to data reported in this paper we shall assume Y is absent and the free oxygen is negligible ($X_F^S = X_F^L = 0$). Thus we write eq. (15) as

$$d\ln a_T = (\bar{W}^S/\bar{W}^L) \, d\ln a_T$$

$$+ (\bar{W}^S/\bar{W}^L)\bar{A}^L \, d\ln a_A + (\bar{X}_B^L - \bar{X}_B^S) \, d\ln p_X . \quad (16)$$

We may simplify this result by first recognizing certain features that exist between the chemical potentials or their activities in the liquid phase through the liquid phase Gibbs-Duhem equation:

$$d\ln a_M = -\bar{X}_B^L \, d\ln p_X - \bar{A}^L \, d\ln a_A - \bar{W}^L \, d\ln a_W . \quad (17)$$

Then the difference between this equation and the result obtained by restricting eq. (13) to X ligation gives

$$d\ln a_T = -\bar{A}^L \, d\ln a_A - \bar{W}^L \, d\ln a_W . \quad (18)$$

When this result is substituted into the right hand side of eq. (16) we obtain the particularly simple form that relates the total hemoglobin activity to the water activity and X ligation:

$$d\ln a_T = -\bar{W}^S \, d\ln a_W + (\bar{X}_B^L - \bar{X}_B^S) \, d\ln p_X . \quad (19)$$

If we assume that $\bar{W}^S$ is constant over the range of water activities that we are interested in then we may integrate eq. (19) and introduce the appropriate partition functions for the oxygen binding to the hemoglobin in liquid and solid phases, Q^L and Q^S respectively, to obtain

$$\ln a_T/a_T^0 = -\bar{W}^S \ln a_W/a_W^0 + \ln Q^L/Q^S . \quad (20)$$

In this expression a_T^0 is the hemoglobin activity (solubility) in the absence of oxygen and added macromolecule A, and a_W^0 is the water activity under the same conditions. The water activity a_W is effected by both hemoglobin and macromolecule A and is measured in a practical way by osmotic pressure or vapor pressure.

5. Discussion

The theoretical analysis of the preceding section can be applied to two sets of experimental measurements. The first example is given by the effect of bovine serum albumin upon the solubility of oxygenated sickle cell hemoglobin, as investigated by Behe and Englander [16]. The second situation is afforded by the experimental measurements we have obtained on the solubility of sickle cell hemoglobin in the presence of bovine serum albumin at different oxygen partial pressures.

The total hemoglobin activity is represented by $a_T = \gamma c_T^L$ where the activity coefficient γ is determined by both the hemoglobin concentration c_T^L and the serum albumin concentration c_A^L in grams per ml solution. Ross and Minton [10] have shown how the total concentration of hemoglobin plus serum albumin may be used to estimate the activity coefficient γ and the water activity a_W. The results are given in terms of the total concentration $c (c = c_T^L + c_A^L)$ in g/ml as follows:

$$\ln a_W = -\frac{18.00}{68000}\,[c + 3.84\,c^2 + 9.216\,c^3 + 16.25\,c^4 + 23.98\,c^5 + 32.23\,c^6 + 44.25\,c^7]\,, \tag{21}$$

$$\ln \gamma = 6.93\,c + 10.95\,c^2 + 14.77\,c^3 + 17.28\,c^4 + 20.74\,c^5 + 27.52\,c^6\,. \tag{22}$$

These equations were obtained from the analysis of Ross and Minton [8] of the non-ideal behaviour of hemoglobin. The additional information needed to calculate the solubility of sickle cell hemoglobin in the presence of albumin is the value of $\bar{W}^S$ and the solubility of unligated sickle cell hemoglobin under standard conditions (0.15 M phosphate, pH 7, 25°C). From the determinations of Sunshine et al. [17], $\bar{W}^S$ is 2400 moles water per mole hemoglobin and the solubility of sickle cell hemoglobin under these standard conditions is 0.19 g/ml [22]. The computed solubilities of deoxygenated sickle cell hemoglobin as a function of serum albumin concentration are shown in fig. 3 for two situations, one where the water content of the solid is assumed zero, and the other with the water content $\bar{W}^S = 2400$. The points obtained by Behe and Englander are plotted in this figure. The

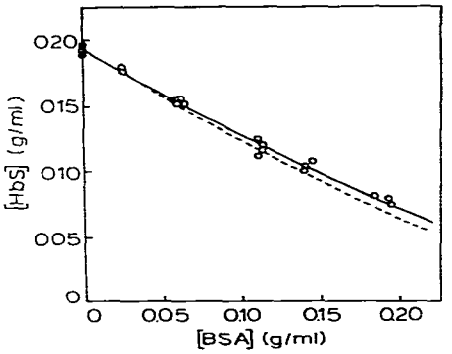

Fig. 3. Solubility of deoxy HbS as a function of BSA concentration. Data points are taken from Behe and Englander [16]. Theoretical calculations represented by solid line is where water content of solid phase (0.7 g HbS/ml) is recognized, and by dashed line is where water content of solid phase is ignored.

calculation assuming zero water content in the solid is similar to that obtained by Ross and Minton [10] who assumed there was no water activity effect upon the water content of the solid phase. One sees that the effect of bovine serum albumin is accurately predicted from the non-ideal solution behavior analogous to that observed for concentrated hemoglobin solutions and from the assumption that serum albumin is excluded from the gel phase. Of particular significance is the fact that although the activity, a_T, of the hemoglobin in equilibrium with the gel phase is increased with increased activity of BSA, the influence of the increased activity coefficient is such as to reduce the equilibrium concentration of the dissolved hemoglobin. If the solution were ideal, just the opposite effect would be predicted. This suggests that there must be a variety of intermediate situations that would depress or enhance the solubility depending upon the degree of non-ideality and exclusion of the added component.

The effect of a ligand, in our case oxygen, brings into play the way in which specific binding affects the solubility of a macromolecule, hemoglobin, in the presence of non-binding macromolecules. One must now include the binding polynomials that express the binding of oxygen to the dissolved and solid (gel) phase hemoglobin. The binding polynomial for the dissolved hemoglobin is expressed in terms of the four

stepwise addition Adair constants, K_1, K_2, K_3, and K_4. These are obtained by a non-linear least square fit on binding curves from solutions of HbS, at non-gelling concentrations, in the presence of BSA, as shown in fig. 1. At this time the oxygen binding curve to the solid phase cannot be determined with high precision, and is approximately represented by a single binding constant, K^S [2,22]. The use of these binding constants in conjunction with eq. (20) enables the activity a_T of the dissolved hemoglobin to be evaluated as a function of oxygen partial pressure. The inclusion of BSA affects values of a_T and a_W as already noted for the calculation of the solubility of unligated hemoglobin solutions. In order to translate the activity a_T to concentration we utilize the activity coefficient expression given by eq. (22). The results of a typical set of such calculations are shown in fig. 4 at different concentrations of BSA. The enhanced solubility at higher oxygen partial pressures or degree of oxygen ligation in the liquid phase is a direct consequence of the greater oxygen affinity to the dissolved over the gelled hemoglobin.

Our data on the amount of birefringence that develops as the oxygen partial pressure is reduced provides a more extensive experimental test of the theoretical equations. However the physical situation is more complicated. In a given experiment the total concentration of hemoglobin and serum albumin is fixed. As more and more gel is formed, however, the concentration of the serum albumin increases in the liquid phase due to its exclusion from the solid phase. The amount of gel formed and the change in the serum albumin concentration can be calculated at given values of the hemoglobin solubility as outlined in the experimental section. The predicted solubility curves (solid lines) for this situation of fixed total amounts of macromolecular components are shown in fig. 2. Also in this figure are shown the points of solubility at different oxygen partial pressures for corresponding experiments with given initial concentrations of BSA. In fig. 5 we show the calculated dependence of BSA concentration on the amount of gel formed. This is seen to increase with lowering oxygen saturation ($\bar{O}_2^L$). In this more complex situation there is still reasonable agreement between the experimentally derived solubility points and the predicted solubility curves. The agreement can be improved by choice of a lower value for the gel concentration (0.5 g/ml) along with a value of higher oxygen affinity to the gel (1.5×10^{-2} $Torr^{-1}$). However, in view of the nature of the current experiments and theoretical calculations, the agreement shown in fig. 2 seems to be as reasonable as might be expected. It should also be pointed out that this case of fixed total amounts of macromolecular components, as is illustrated by the results in fig. 2,

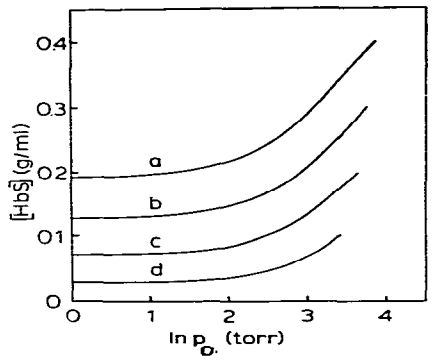

Fig. 4. Calculated solubilities of HbS as a function of the logarithm of the oxygen partial pressure (p_O) at fixed BSA concentrations: a) 0.0 g BSA/ml, b) 0.1 g BSA/ml, c) 0.2 g BSA/ml, d) 0.3 g BSA/ml. The initial concentrations of HbS were taken as follows: a) 0.4 g HbS/ml, b) 0.3 g HbS/ml, c) 0.2 g HbS/ml, and d) 0.1 g HbS/ml.

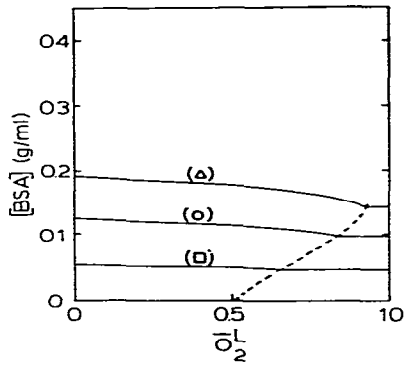

Fig. 5. Calculated concentration of BSA versus extent of oxygenation in the liquid phase ($\bar{O}_2^L = \bar{X}^L$) as gelation of HbS proceeds under specified initial BSA concentrations given in fig. 2. The curves are designated by the appropriate symbols corresponding to the conditions of fig. 2. The dashed line indicates where gelation initially occurs.

coincides with the expected physical situation within the erythrocyte containing sickle cell hemoglobin and additional non-gelling, non-hybrid forming hemoglobin.

The general success of the theoretical predictions and the observed solubilities for this case of the effect of a totally excluded macromolecule from the gel phase motivates some discussion of the possible effects of partially excluded non-binding molecules from the gel phase. In this case the coefficient of d ln a_A in eq. (15) will be a fraction of the total exclusion case. The interstitial space available to A in the solid phase will be less than that available to it in the liquid phase. The extent of availability will depend upon the size of A in comparison with the maximum dimensions and specific shape of the interstitial space. For small molecules of A, i.e. the size of water molecules, we expect equal partitioning and no effect upon the activity of a_T. From the size of hemoglobin of approximate diameter of 50 Å, one expects the interstitial space in crystals to be capable of excluding molecules of the order of 10 Å to a reasonable extent. One would predict in this situation that molecules such as di- or tripeptides would be partially excluded, and if present in sufficient concentrations, should produce enhanced solubility of crystalline hemoglobin. The interstitial space in sickle cell hemoglobin gels is larger due to the smaller hemoglobin concentration so the exclusion effect will be reduced. Of course, even greater enhancement will occur when specific binding differences between solid and liquid forms exist, but the exclusion effect is quite general. This, perhaps explains the wide class of antisickling agents that have been found [23]. Many of these show a general tendency that the larger the molecule the greater the enhancement of the solubility of the sickle cell hemoglobin.

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References

[1] S.J. Gill, R.C. Benedict, L. Fall, R. Spokane and J. Wyman, J. Mol. Biol. 130 (1979) 175.
[2] S.J. Gill, R. Spokane, R.C. Benedict, L. Fall and J. Wyman, J. Mol. Biol. 140 (1980) 299.
[3] J. Wyman and S.J. Gill, Proc. Natl. Acad. Sci. U.S.A. 77 (1980) 5239.
[4] A.P. Minton, J. Mol. Biol. 82 (1974) 483.
[5] A.P. Minton, J. Mol. Biol. 95 (1975) 289.
[6] A.P. Minton, J. Mol. Biol. 100 (1976) 519.
[7] A.P. Minton, J. Mol. Biol. 110 (1977) 89.
[8] P.D. Ross and A.P. Minton, J. Mol. Biol. 112 (1977) 437.
[9] G.S. Adair, Proc. Roy. Soc. Ser. A, 120 (1928) 573.
[10] P.D. Ross and A.P. Minton, Biochem. Biophys. Res. Comm. 88 (1979) 1308.
[11] D. Dolman and S.J. Gill, Anal. Biochem. 87 (1978) 127.
[12] D.L. Drabkin and J.H. Austin, J. Biol. Chem. 112 (1935) 51.
[13] E. Antonini and M. Brunori, Hemoglobin and myoglobin in their reactions with ligands (North-Holland, Amsterdam, 1971).
[14] J. Hofrichter, P.D. Ross and W.A. Eaton, Proc. Natl. Acad. Sci. U.S.A. 73 (1976) 3035.
[15] J.F. Bertles, R. Rabinowitz and J. Dobler, Science 169 (1970) 375.
[16] J.J. Behe and S.W. Englander, Biophys. J. 23 (1978) 129.
[17] H.R. Sunshine, J. Hofrichter and W.A. Eaton, J. Mol. Biol. 133 (1979) 435.
[18] J. Wyman, Advan. Protein Chem. 19 (1964) 223.
[19] S.J. Gill, in: Biochemical thermodynamics, ed. M.N. Jones (Elsevier Scientific Publishing Co., Amsterdam, 1979) pp. 224–255.
[20] J.A. Schellman, Biopolymers 14 (1975) 999.
[21] C. Tanford, J. Mol. Biol. 39 (1969) 539.
[22] H.R. Sunshine, F.A. Ferrone, J. Hofrichter and W.A. Eaton, Fed. Proc. 39 (1980) 2195.
[23] P.D. Ross and S. Subramanian, in: Biochemical and clinical aspects of hemoglobin abnormalities, ed. W.S. Caughey (Academic Press, New York, 1977) pp. 629–646.