Deoxygenated Sickle Hemoglobin

EFFECTS OF LYOTROPIC SALTS ON ITS SOLUBILITY*

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The effects of 22 lyotropic salts on the polymerization of deoxygenated sickle hemoglobin (deoxy-Hb S) were evaluated. The equilibrium solubility was measured as the saturation concentration, \( c_{\text{sat}} \), after phase separation by centrifugation at 30°C. The linear plots of \( c_{\text{sat}} \) versus salt concentration allowed the assignment of particular cations or anions as either salting-in (\( c_{\text{sat}} \) increased) or salting-out (\( c_{\text{sat}} \) decreased) agents. Divalent cations were considerably more effective than univalent cations as salting-in agents. Overall, the effects of cations on the solubility of deoxy-Hb S were: \( \text{Ca}^{2+} > \text{Mg}^{2+} > \text{NH}_4^+ > \text{Na}^+ > \text{K}^+ > \text{Li}^+ > \text{Rb}^+ > \text{K}^+ \). Univalent anions in the presence of the counter cation \( \text{Na}^+ \) were, with the exception of \( \text{F}^- \), salting-in agents which followed the order of the Hofmeister series (Hofmeister, F. (1888) Arch. Exp. Pathol. Pharmakol. 24, 247-260): \( \text{SCN}^- > \text{ClO}_4^- > \text{I}^- > \text{Br}^- > \text{Cl}^- \). Such differential effects were not observed, however, in the presence of the guanidinium cation. Instead, all four guanidinium salts evaluated were potent salting-in agents and increased \( c_{\text{sat}} \) to the same extent regardless of the counter anion (\( \text{SCN}^- \), \( \text{Cl}^- \), \( \text{NO}_2^- \), \( \text{SO}_4^{2-} \)). This uniformity of response most likely results from the extremely potent salting-in capacity of the guanidinium cation, which completely overweights any expression of the counter anion. Three anions (\( \text{F}^- \), \( \text{PO}_4^{3-} \), and \( \text{SO}_4^{2-} \)) behaved overall as salting-out agents whose molar effectiveness was: \( \text{SO}_4^{2-} > \text{HPO}_4^{2-} > \text{F}^- \). In general, the magnitude and the direction of the effect of salts on solubility depend on the specific ions involved. Moreover, cations and anions act in roughly additive fashion.

The in vitro correlate of such intracellular polymerization is the gelation of hemolysates of SS erythrocytes (or purified Hb S) observed when the intrinsic solubility of deoxy-Hb S is exceeded (3, 4). Both the kinetics and the thermodynamics of the nucleation controlled polymerization process involved in sickling have been studied by a variety of physical techniques over the past several years (3-7).

Magdoff-Fairchild et al. (3) first used the ultracentrifugation technique of Bertles et al. (8) to evaluate the solubility of deoxy-Hb S under varying conditions of pH, temperature, and initial concentration. Their studies showed that solubility could be measured as the saturation concentration, \( c_{\text{sat}} \), above which an equilibrium mixture of monomers and polymers exists. This technique has subsequently been used by others (9-11) to evaluate the effects of potential anti-sickling agents on the solubility of deoxy-Hb S.

Since hydrophobic forces are known to be strengthened by increasing temperature (12), the negative temperature coefficient of the gelation of deoxy-Hb S, first described by Murayama (13), implicates hydrophobic bonding as the predominant noncovalent interaction which stabilizes the polymeric fiber. Other lines of evidence suggest that hydrogen bonds (14) and electrostatic interactions (15, 16) may contribute as well.

Recent studies of the effects of neutral salts on the conformational stability of a wide variety of biological macromolecules (17, 18) indicate that such electrolytes perturb the balance of stabilizing forces which maintain the "native" state by some mechanism other than general electrostatic shielding. Rather, lyotropic salts exert specific effects which are determined solely by the nature of the salt. Accordingly, the present study was undertaken in order to provide a systematic evaluation of the effects of neutral electrolytes on the solubility of deoxy-Hb S.

MATERIALS AND METHODS

Sickle Hemoglobin—Venous blood, anticoagulated with EDTA, was obtained from a single individual over the course of 10 months. The erythrocytes from this particular individual have a sufficiently low fetal hemoglobin (Hb F) content (<2%) to obviate any consideration of the well known interaction of Hb S and Hb F (8, 19, 20) on the solubilities observed. Erythrocytes were washed and lysed as previously described (3). Hemolysates were concentrated to 32 to 36 g/dl by vacuum ultrafiltration, followed by extensive dialysis against 0.05 M bis-Tris buffer (pH 6.80 at 30°C) composed of equimolar amounts of K\(_2\)HPO\(_4\) and KH\(_2\)PO\(_4\). (This buffer will be referred to as K\(_2\)HPO\(_4\), in the appropriate tables and figures.)

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1. The abbreviations used are: Hb S, sickle hemoglobin; \( c_{\text{sat}} \), saturation concentration; MGC, minimum gelating concentration; GdnHCl, guanidinium cation; bis-Tris, [bis(2-hydroxyethyl)amino]tris(hydroxymethyl)methane; SS erythrocytes, erythrocytes from patients homozygous for Hb S; Hb F, fetal hemoglobin.

2. Because cell-free hemolysates, rather than purified Hb S, were employed throughout, the use of SS erythrocytes from a single individual was essential to avoid undue variations in solubility which would result had blood from several patients been used.

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Neutral Electrolytes—The best quality reagent grade salts were obtained from various commercial sources and used without further purification. Those which were obviously deliquescent were dried to constant weight in vacuo over \( \text{P}_{2}\text{O}_{5} \) prior to use. Concentrated stock solutions (usually 2 to 4 m) were prepared in distilled water.

**Solubility Measurements**—Equilibrium solubility, measured as \( c_{\text{sol}} \), was determined as previously described (3), with the following modifications. Appropriate aliquots of dialyzed Hb S were placed in micro-sized centrifuge tubes (5 x 42 mm) together with suitable admixtures of stock salt solution and distilled water so as to encompass the concentration range of interest for any particular salt. The appropriate concentration of phosphate was achieved by admixture of the requisite amount of 2 M KPO\(_4\) buffer, pH 6.80, with distilled water. Samples of 400 \( \mu \)l total volume were then treated as described elsewhere (3). After centrifugation, the pH of the supernatant was measured through mineral oil with an Ingold combination microelectrode and a Radiometer model 26 pH meter. In no case did the measured pH deviate by more than \( \pm 0.20 \) from the nominal value of 6.80. This pH was chosen because it is close to the isoelectric point of Hb S and is midway in the pH region (6.4 to 7.1) where the solubility of deoxy-Hb S is both minimal and nearly invariant (3, 19). The initial concentration of Hb S was varied over the range 12 to 30 g/dl, depending upon the particular salt to be used.Because of variable dilution incurred by the addition of aqueous salt solutions, the final concentration of bis-Tris buffer ranged from 38 to 42 mm. The solubility profile obtained for each salt allowed the assignment of particular ions as either salting-in or salting-out agents. For ions with salting-in properties, \( c_{\text{sol}} \) was increased relative to the control, while for those with salting-out properties, \( c_{\text{sol}} \) was decreased. Cation effects were evaluated in the presence of the counter anion Cl\(^-\), while anion effects were evaluated in the presence of various counter cations.

**RESULTS**

**Base-line Solubilities**—Overall, the effects of 22 lyotropic salts on the solubility, \( c_{\text{sol}} \), of deoxy-Hb S were evaluated. In each experiment, a control sample (in the absence of salt) was included. For the 21 neutral electrolytes of deoxy-Hb S were evaluated. In each experiment, a control sample (in the absence of salt) was included. For the 21 neutral electrolytes of deoxy-Hb S were evaluated. In each experiment, a control sample (in the absence of salt) was included. For the 21 neutral electrolytes of deoxy-Hb S were evaluated. In each experiment, a control sample (in the absence of salt) was included. For the 21 neutral electrolytes of deoxy-Hb S were evaluated. In each experiment, a control sample (in the absence of salt) was included. For the 21 neutral electrolytes of deoxy-Hb S were evaluated. In each experiment, a control sample (in the absence of salt) was included. The 21 neutral electrolytes of deoxy-Hb S were evaluated. 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Effects of Lyotropic Salts on the Solubility of Deoxy-Hb S

Fig. 3. Salting-out effects of various salts on the equilibrium solubility of deoxy-Hb S. Other experimental conditions are given in the legend to Fig. 1.

Considerations of 0.2 to 0.4 M, however, c sat decreased relative to the control in all cases. Such complex solubility profiles show that salting-in (region of positive slope) precedes salting-out (region of negative slope). Furthermore, since salting-in occurs at low concentrations and is most pronounced for MgSO4, it can very likely be attributed to the cation. That is, the divalent cation Mg2+ is a much more effective salting-in agent than any of the univalent cations (Fig. 1). By similar reasoning, the eventual predominance of salting-out at high concentrations indicates the influence of the anion. Inasmuch as we are primarily interested in salting-out, the results of Fig. 3 have been interpreted only in terms of anion effects. The slopes of the various solubility plots presented in Figs. 1 to 3 have been calculated and are compiled in Table I.

Thermodynamic Analysis of Solubility Data—The polymerization of deoxy-Hb S under conditions of thermodynamic equilibrium may be depicted thus:

\[ n\text{Hb} \rightleftharpoons (\text{Hb})_n \]

The perturbation of this equilibrium by the electrolytes examined is reflected by changes in solubility measured as \( c_{\text{sat}} \), ions with salting-in properties inhibit polymerization while those with salting-out properties enhance it.

The overall free energy change, \( \Delta G_{\text{depoly}} \), of depolymerization at 30°C for any particular salt at 0.2 M concentration is given by:

\[ \Delta G_{\text{depoly}} = -1.389(\log c_{\text{sat}} + 0.2 K_s) \quad (1) \]

where \( K_s \), the Setschenow constant, is a solubility parameter.
reflecting the direction and magnitude of the lyotropic effect exerted by any given salt. (The derivation of this equation can be found in the miniprint supplement which follows this article.)

Values of $\Delta G_{\text{cat}}$ were calculated from Equation 1 for each of the 22 salts evaluated and are plotted in Fig. 4 versus the corresponding value of $K_c$. This relationship conveniently illustrates the thermodynamic distinction between salting-in and salting-out electrolytes. Those lyotropic salts with salting-in properties have values of $K_c < 0$ while those with salting-out properties have values of $K_c > 0$. (The point at which $K_c = 0$ is equivalent to $\Delta G_{\text{cat}}$, the free energy change associated with depolymerization in the absence of salt, and has a value of 3.54 kcal/mol.) The range of $\Delta G_{\text{cat}}$ varies from 3.13 kcal/mol for the most effective salting-in agent (CaCl$_2$) to 3.87 kcal/mol for the most effective salting-out agent (Na$_2$SO$_4$). These two extremes are separated by only 0.74 kcal/mol, indicative of the sensitivity of the molecular architecture of the deoxy-Hb S polymer to perturbations by lyotropic salts.

**DISCUSSION**

A schematic diagram showing the effects of various ions on the solubility of deoxy-Hb S is depicted in Table II. These individual ion rankings, considered in conjunction with the data summarized in Table I, allow the following generalizations to be made.

1. **Divalent cations (Ca$^{2+}$, Mg$^{2+}$) are considerably more effective salting-in agents than univalent cations (Fig. 1). In fact, the latter are, for the most part, relatively weak salting-in agents whose effects on $c_{\text{sat}}$ tend to level off above concentrations of about 0.4 M.**

2. **Univalent anions in the presence of the counter cation Na$^+$ are, with the exception of F$^-$, salting-in agents whose order rank follows that of the Hofmeister series (21) (Fig. 1). Consideration of the order observed for the halide anions shows that Cl$^-$ has effectively neither salting-in nor salting-out properties. That is, it occurs approximately at the cross-over point between these two opposing effects, i.e. where $K_c$ changes sign (Fig. 4).**

3. **Both divalent anions (SO$_4^{2-}$ and HPO$_4^{2-}$) evaluated, as well as one univalent anion (F$^-$), were salting-out agents at concentrations in the range 0.2 to 1.0 M (Fig. 3).**

4. **Anion and cation effects are exerted individually. That is, for any given ion pair, the anion modulates the expression of the cation and vice versa. Thus, the molar effectiveness of the SO$_4^{2-}$ anion as a salting-out agent was determined by its counter cation (Table I) according to the following order:**

\[
\text{Na}_2\text{SO}_4 > \text{Cs}_2\text{SO}_4 > (\text{NH}_4)_2\text{SO}_4 > \text{MgSO}_4.
\]

By contrast, the efficacy of the chloride salts of these same cations as salting-in agents followed the reverse order:

\[
\text{MgCl}_2 > \text{NH}_4\text{Cl} > \text{CsCl} > \text{NaCl}.
\]

The thermodynamic analysis of the solubility data is presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 3650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 78M-1265, cite author(s), and include a check or money order for $1.00 per set of photocopies.

This behavior substantiates the view that the ions of a particular salt exert their effects independently, in essentially additive fashion. The generality of this principle of additivity has been amply demonstrated in studies by others on the effects of neutral salts on the conformational stability of widely diverse macromolecular structures (24).

5. **The effect of guanidinium salts on the solubility of deoxy-Hb S is remarkable in that a uniform increase in $c_{\text{sat}}$ was observed regardless of the accompanying anion (Fig. 2). Inasmuch as three of the four anions examined (SCN$^-$, Cl$^-$, and SO$_4^{2-}$) showed widely different effects on $c_{\text{sat}}$ when evaluated in the presence of the counter cation Na$^+$ (Figs. 1 to 3), such behavior was totally unexpected. This was especially so in light of the fact that for two other grossly different systems, the isothermal unfolding of ribonuclease (25) and critical micelle formation for the nonionic detergent Triton X-100 (26), the companion anion exerted significant modulating effects when these same guanidinium salts were evaluated as perturbants of macromolecular stability. It would appear, therefore, that the lack of expression of anion effects in the presence of GdnHCl is unique to the deoxy-Hb S system.**

In light of the additivity of anion and cation effects on solubility discussed previously, the uniform effect of the guanidinium salts on the solubility of deoxy-Hb S clearly implicates the cation as the agent responsible for this behavior. While it is not possible to make a definitive interpretation of this anomaly, it is unlikely that the guanidinium salts act via the nonspecific solvent-mediated effect which pertains in protein denaturation exhibited at high concentrations (>5 M) of GdnHCl (27). Such an interpretation is unsatisfactory for the deoxy-Hb S system since it cannot account for the lack of an anion effect. Rather, it is more likely that disruption of one or more specific ion bonds in the polymer occurs due to an electrostatic interaction with the guanidinium cation. This is especially likely since such low concentrations of guanidinium salts (<0.2 M) are needed to produce this solubilizing effect.

Since the guanidinium moiety corresponds to the side chain functional group of the amino acid arginine, GdnH$^+$ could competitively inhibit the formation of one or more salt bridges involving arginine at regions of intermolecular contact in the polymer. Such a specific electrostatic interaction would account for the lack of expression of anion effects.

Levine and others (29, 30) have evaluated the effects of representative examples of the Hofmeister anions (21) on the solubility of deoxy-Hb S measured both by MGC and by salting-out in strong phosphate buffer. In accordance with our results (Figs. 1 and 2), the effectiveness of these anions either in inhibiting gelation or in increasing the solubility in 1.96 M KPO$_4$ buffer, pH 7.0, followed the order:

\[
\text{SCN}^- > \text{Br}^- > \text{NO}_3^- > \text{Cl}^-
\]

It is reassuring to note this consistency of response to anion effects as measured by the two physiologically relevant kinds of solubility assay (MGC and $c_{\text{sat}}$). Regardless of whether one monitors gelation or saturation concentration, it would appear that both assays respond to the inhibition of polymerization in similar fashion.

The participation of ionic bonds in maintaining the structural integrity of the deoxy-Hb S polymer has been inferred.

A study germane to this issue was that of Allison (28), who showed that the large increment of viscosity observed when concentrated solutions of Hb S were deoxygenated was abolished in the presence of 0.5 M guanidinium chloride.

For solubilities measured by the latter method, very high concentrations of phosphate (2 to 5 M) are used. Such solubilities ought not to be confused with those depicted in Fig. 3, which require only moderate concentrations (<1 M) of phosphate.

**TABLE II**

<table>
<thead>
<tr>
<th>Counter ion</th>
<th>Order of increasing solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl$^-$</td>
<td>K$^+$, Rb$^+$ &lt; Na$^+$, Cs$^+$ &lt; Li$^+$ &lt; NH$_4^+$ &lt; Mg$^{2+}$ &lt; Ca$^{2+}$</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>SO$_4^{2-}$ &lt; HPO$_4^{2-}$ &lt; F$^-$ &lt; Cl$^-$ &lt; Br$^-$ &lt; I$^-$, ClO$_4^-$ &lt; SCN$^-$</td>
</tr>
<tr>
<td>GdnH$^+$</td>
<td>SO$_4^{2-}$, NO$_3^-$, Cl$^-$, SCN$^-$</td>
</tr>
</tbody>
</table>

*The effect of guanidinium salts on the solubility of deoxy-Hb S is depicted in Table II. These individual ion rankings, considered in conjunction with the data summarized in Table I, allow the following generalizations to be made.*

*1. Divalent cations (Ca$^{2+}$, Mg$^{2+}$) are considerably more effective salting-in agents than univalent cations (Fig. 1). In fact, the latter are, for the most part, relatively weak salting-in agents whose effects on $c_{\text{sat}}$ tend to level off above concentrations of about 0.4 M.*

*2. Univalent anions in the presence of the counter cation Na$^+$ are, with the exception of F$^-$, salting-in agents whose order rank follows that of the Hofmeister series (21) (Fig. 1). Consideration of the order observed for the halide anions shows that Cl$^-$ has effectively neither salting-in nor salting-out properties. That is, it occurs approximately at the cross-over point between these two opposing effects, i.e. where $K_c$ changes sign (Fig. 4).*

*3. Both divalent anions (SO$_4^{2-}$ and HPO$_4^{2-}$) evaluated, as well as one univalent anion (F$^-$), were salting-out agents at concentrations in the range 0.2 to 1.0 M (Fig. 3).*

*4. Anion and cation effects are exerted individually. That is, for any given ion pair, the anion modulates the expression of the cation and vice versa. Thus, the molar effectiveness of the SO$_4^{2-}$ anion as a salting-out agent was determined by its counter cation (Table I) according to the following order:*

\[
\text{Na}_2\text{SO}_4 > \text{Cs}_2\text{SO}_4 > (\text{NH}_4)_2\text{SO}_4 > \text{MgSO}_4.
\]

*By contrast, the efficacy of the chloride salts of these same cations as salting-in agents followed the reverse order:*

\[
\text{MgCl}_2 > \text{NH}_4\text{Cl} > \text{CsCl} > \text{NaCl}.
\]
from the increased MGC observed at high concentrations of NaCl (14, 15). However, it may be seen from Table I that NaCl is a relatively weak salting-in agent and therefore a poor choice as a source of ionic strength. Furthermore, in light of the fact that both the magnitude and the direction of the effect on solubility depend on the specific ions involved (Table I), a general electrostatic shielding effect cannot be operative in this case. Instead, some mechanism affecting those nonpolar groups which comprise regions of intermolecular contact in the polymer should be considered to explain the specificity observed.

Considerable effort has been expended by others (22, 31–34) to explain how anions perturb the stability of widely dissimilar biological macromolecules according to the ranking of the Hofmeister series (21). These studies have used model compounds containing both peptide and hydrophobic components to represent the interaction of a typical protein amino acid residue with the solvent. In each case, the solubility behavior could be explained by separating salt-induced interactions into polar and nonpolar components. The specificity of the lyotropic salts could then be accounted for by a fairly constant, nonpolar salting-out effect on the peptide component (direct binding of the ion-dipole type) and a specific salting-out effect on the hydrophobic component. Thus, according to this theory, the specificity of the Hofmeister series (21) arises solely from the magnitude of the salting-out coefficients for the nonpolar groups, the polar groups all being salted-in to an extent dependent only on ionic charge. Another explanation of the specificity of the Hofmeister series has recently been presented by Melander and Horvath (35) who showed that measured salting-out constants for horse HbCO (36) could be represented by the sum of two mutually opposing terms, an electrostatic salting-in coefficient and a hydrophobic salting-out coefficient. The novel feature of this treatment was that it related the solubility of surface hydrophobic residues to a specific salt effect on the surface tension of water. This is particularly important in offering an explanation for salting-out behavior at high salt concentrations, for which the rationale based on the solubility of model peptides is unsatisfactory. Regardless of which of the above approaches is used, it is the hydrophobic component of the protein to which the specificity of the Hofmeister anions must be attributed. In our case, even though the salt concentrations shown in Fig. 2 are considerably lower (<0.5 M) than those which are effective in perturbing the conformational stability of other biopolymers, a similar rationale may be invoked. That is, because there is less stabilization energy to overcome (3 to 4 kcal/mol) than in the dissociation of Hb A (6 to 7 kcal/mol, Ref. 37) or the isothermal unfolding of ribonuclease (10 to 20 kcal/mol, Ref. 38), lower concentrations of salt are sufficient to perturb the stabilizing noncovalent interactions.

The individual ion rankings demonstrated in this study rule out a general, ionic strength mechanism as the sole basis for the effect of various salts on the polymerization of deoxy-Hb S. Inasmuch as hydrophobic interactions play the predominant role in stabilizing the supramolecular structure of the deoxy-Hb S polymer (13, 39, 40), it is very likely that lyotropic salts exert their effects at least as much by perturbation of such forces as by nonspecific electrostatic shielding.

Acknowledgment—We are grateful to Dr. B. Magdoff-Fairchld for aid in the linear regression analyses of the solubility data by computer.

REFERENCES

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SUPPLEMENTARY MATERIAL

Deoxygenated Sickle Hemoglobin:

Effects of Lyotropic Salts on Its Solubility

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Thermodynamic Analysis of Solubility Data. The polymerization of deoxy-Hb S under conditions of thermodynamic equilibrium may be depicted thus:

\[ \text{nHb} \rightleftharpoons (\text{Hb})_n \] (1)

The effect of any salt on its solubility can be analyzed according to the Setschenow equation:

\[ \log \left( \frac{S_0}{S} \right) = K_s c_s \] (2)

where \( S_0 \) and \( S \) represent the respective molar solubilities of deoxy-Hb S in the absence or presence of salt at any given concentration, \( c_s \), and \( K_s \) is the Setschenow constant. A measure of the extent to which the equilibrium depicted in Equation (1) is perturbed by the added electrolyte is given by:

\[ \Delta G_{\text{salt}} = RT \ln \left( \frac{S_0}{S} \right) \] (3)

Furthermore, so long as plots of \( \log \left( \frac{S_0}{S} \right) \) vs. \( c_s \) are linear (22), \( \Delta G_{\text{salt}} \) and \( K_s \) are directly related by:

\[ \Delta G_{\text{salt}} = 2.303 RT K_s c_s \] (4)

where \( R \) is the molar gas constant and \( T \) the absolute temperature. This free energy difference represents the extent to which intermolecular noncovalent forces in the polymer are stabilized (salting out) or destabilized (salting in) for any given perturbant. The overall free energy change associated with the perturbation of the polymer to monomer transition by any particular salt may then be computed according to the following relationship:

\[ \Delta G_{D,\text{salt}} = \Delta G_{D,\text{w}} + \Delta G_{\text{salt}} \] (5)

Since the polymerization of deoxy-Hb S is nucleation controlled (5,23), the equilibrium constant for polymerization corresponds to \( 1/c_{\text{sat}} \), while the equilibrium constant for depolymerization corresponds to \( c_{\text{sat}} \). The first term on the right of Equation (5), the free energy change for depolymerization in the absence of salt, may be evaluated by:

\[ \Delta G_{D,\text{w}} = -RT \ln c_{\text{sat}}^0 \] (6)

where \( c_{\text{sat}}^0 \) is the solubility in the absence of salt under the specified conditions of pH and temperature. Thus both terms on the right of Equation (5) may be evaluated from the solubility data of Figs. 1-3 and the derived values of \( K_s \) compiled in Table I. Inspection of Figs. 1 and 2 shows that, considered in the aggregate, the solubility profiles for the salting-in ions are linear up to about 0.2 M. Accordingly, this salt concentration was chosen as reference for the evaluation of \( \Delta G_{\text{salt}} \). Solubility profiles for the salting-out anions (Fig. 3) are peculiar in that, with the exception of phosphate, a region of salting in precedes that of salting out. Accordingly, these anions have been treated as if the origin corresponded to the maximal value of \( c_{\text{sat}} \), the point at which salting out begins.
Deoxygenated sickle hemoglobin. Effects of lyotropic salts on its solubility.
W N Poillon and J F Bertles


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