How Hofmeister Ion Interactions Affect Protein Stability*

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ABSTRACT Model compound studies in the literature show how Hofmeister ion interactions affect protein stability. Although model compound results are typically obtained as salting-out constants, they can be used to find out how the interactions affect protein stability. The null point in the Hofmeister series, which divides protein denaturants from stabilizers, arises from opposite interactions with different classes of groups: Hofmeister ions salt out nonpolar groups and salt in the peptide group. Theories of how Hofmeister ion interactions work need to begin by explaining the mechanisms of these two classes of interactions. Salting-out nonpolar groups has been explained by the cavity model, but its use is controversial. When applied to model compound data, the cavity model 1) uses surface tension increments to predict the observed values of the salting-out constants, within a factor of 3, and 2) predicts that the salting-out constant should increase with the number of carbon atoms in the aliphatic side chain of an amino acid, as observed. The mechanism of interaction between Hofmeister ions and the peptide group is not well understood, and it is controversial whether this interaction is ion-specific, or whether it is nonspecific and the apparent specificity resides in interactions with nearby nonpolar groups. A nonspecific salting-in interaction is known to occur between simple ions and dipolar molecules; it depends on ionic strength, not on position in the Hofmeister series. A theory by Kirkwood predicts the strength of this interaction and indicates that it depends on the first power of the ionic strength. Ions interact with proteins in various ways besides the Hofmeister ion interactions discussed here, especially by charge interactions. Much of what is known about these interactions comes from studies by Serge Timasheff and his co-workers. A general model, suitable for analyzing diverse ion-protein interactions, is provided by the two-domain model of Record and co-workers.

INTRODUCTION

Hofmeister ion effects on protein stability arise repeatedly in protein research. They account for phenomena such as GdmSCN being a much stronger denaturant than GdmCl, and $(Gdm)_2SO_4$ not being a denaturant at all (von Hippel and Wong, 1964). Students and sometimes colleagues frequently ask: What is the mechanism of action of Hofmeister ions on proteins, and why are some ions stabilizers but other ions denaturants? At a practical level, the answer to this question is given by model compound studies in the older literature, and a main purpose of this review is to summarize the conclusions of these studies. Another purpose is to point out that, when Hofmeister ion interactions are analyzed by use of model compound studies, they show characteristic features: 1) They occur by the weak interaction model, not by the site binding model, and each interaction can be characterized by a salting-out or salting-in constant. 2) They are specific interactions such that the order of the salting-out or salting-in constants defines the Hofmeister series. 3) The effect of the interaction continues to change, as defined by the Setchenow equation, at high salt concentrations, for example, in the range 1–5 M. The terms "Hofmeister ion" and "Hofmeister salt" are used here to mean that Hofmeister interactions, as defined by these characteristic properties, are under discussion, and to distinguish them from nonspecific interactions, such as ionic strength-dependent interactions.

The last purpose is to discuss the use of the cavity model as a semiquantitative explanation for the salting-out of nonpolar molecules by Hofmeister ions. This has been discussed earlier (see Melander and Horvath, 1977). The cavity model is applied here to the problem of predicting values of the salting-out constants measured by Nandi and Robinson (1972a).

A useful summary of Hofmeister ion interactions and how they affect protein stability is given by Jencks (1987). Hofmeister ions are generally thought to exert their effects indirectly by changing the hydrogen-bonding properties of water. The question of how Hofmeister ions affect the structure and hydrogen-bonding properties of water was reviewed by Collins and Washabaugh (1985), and the problem is being actively investigated today; see the neutron diffraction study of the structure of water in the presence of Hofmeister ions (Leberman and Soper, 1995) and the accompanying commentary by Parsegian (1995). This problem is not discussed here.

Hofmeister (1888) defined the series of anions and cations that bears his name when he measured the concentrations of various salts needed to precipitate proteins from whole egg white. Hofmeister's famous paper is the second part of a two-part paper; the first part was written by his student Lewith (1888). Before the advent of protein chromatography, salting out was the major method used to purify proteins. This practical usage provided the rationale for investigating the factors that control salting out of pro-

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*Dedicated to Serge Timasheff on his 70th birthday.

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teins (see Green, 1932) and model compounds. Many of the results are summarized and analyzed in the book by Cohn and Edsall (1943).

RESULTS AND DISCUSSION

Thermodynamic theory

When Hofmeister ion interactions are studied in isolation, using model compounds, salting out follows the empirical equation of Setchenow (1892). He was interested in investigating how salts affect the solubility of CO_2 to understand the transport of CO_2 in the bloodstream. He found that the logarithm of the solubility varies linearly with the salt concentration. His equation can be written:

$$\log[C_i/C_i(0)] = -k_s C_s, \tag{1}$$

where C_i is the molar concentration of the solute under study, C_s is the concentration of the salt, and k_s is the salting-out constant (or coefficient).

Before deriving Setchenow's equation from equilibrium thermodynamics, it is useful to compare the weak interaction and site binding models for the interaction between a Hofmeister salt and the solute. The site binding model treats a Hofmeister ion as a chemical reactant. If r anions (species A) interact with one molecule of solute (i), the site binding model describes their interaction as a chemical reaction:

$$rA + i \rightleftharpoons iA_r \tag{2a}$$

$$r\mu_A + \mu_i = \mu_i A_r \tag{2b}$$

$$\mu_i = \mu_i^\circ + RT \ln y_i C_i \tag{2c}$$

$$\mu_A = \mu_A^\circ + RT \ln y_A C_A, \tag{2d}$$

where μ_i, μ_A are the chemical potentials (or partial molar Gibbs energies) of i and A, the μ° values are reference potentials, and y_i, y_A are the activity coefficients of i and A, and the effect of an electric field on the ions is omitted because the term drops out when the chemical potential of a neutral salt is expressed. This treatment is familiar when the solute binds protons instead of anions, and the appropriate working equations for various practical cases are well known. The weak interaction model treats a Hofmeister salt as a chemical perturbant. The chemical potential of the solute i is written as a function of the salt (component k) concentration as well as of its own concentration,

$$\mu_i = \mu_i^\circ + RT \ln y_i C_i + RT\beta_i + \ldots \tag{3}$$

$$\beta_i = \beta_{ik} C_k + \ldots \tag{3a}$$

$$\beta_{ik} = \frac{\partial \mu_i}{\partial C_k}, \tag{3b}$$

where $RT\beta_i$ is the excess free energy (see Schellman, 1990). This term, resulting from the interaction between the solute i and the salt k, is expressed by a Maclaurin's series (the derivative β_{ik} is evaluated at $C_k = 0$), and usually the first term in the series is found to be sufficient. At low solute concentrations, the activity coefficient y_i can be set equal to 1.

The weak interaction model given in Eq. 3 applies to the case when an uncharged molecule i interacts with a salt k. In studies of proteins, interactions with salt ions arise directly from the charge on the protein and they must also be considered. A general thermodynamic model suitable for this purpose, the "two-domain model," has been developed by Anderson and Record (1993). Applications of the two-domain model to cases including Hofmeister ion interactions are currently in progress (M. T. Record, personal communication).

Setchenow's equation is derived from Eq. 3 by noting that μ_i remains constant as C_k is varied in a salting-out study when solute i is in equilibrium between the solution and the solid phase. Then Eq. 1 is obtained with

$$k_s = \beta_{ik}/2.303. \tag{4}$$

The effect of the salt (component k) on the equilibrium N $\rightleftharpoons$ U between native protein (N) and unfolded protein (U) is obtained by writing Eq. 3 separately for N and U and then noting that $\mu_N = \mu_U$ at equilibrium. The result is

$$-RT \ln K_{NU} = \Delta G^\circ_{NU} + RTC_k(\beta_{Nk} - \beta_{Uk}) \tag{5}$$

$$K_{NU} = C_N/C_U \tag{5a}$$

$$\Delta G^\circ_{NU} = \mu^\circ_N - \mu^\circ_U. \tag{5b}$$

Equation 5 has the same form as the linear extrapolation method for obtaining the standard free energy of protein unfolding at $C_k = 0$ when k is a denaturant (urea or GdmCl) (Pace and Vanderburg, 1979; Santoro and Bolen, 1988). The weak interaction model can be derived by considering contact interactions between the denaturant and the protein, and by taking account of the exchange between the denaturant and water at the contact site (Schellman, 1987, 1990). Because there is still vigorous discussion today about whether the weak interaction model or the site binding model should be used to describe the interaction of urea or GdmCl with a protein (see Makhatadze and Privalov, 1992), it is important to note that the validity of the weak interaction model for describing Hofmeister ion interactions with model compounds is tested directly by determining whether the Setchenow equation is satisfied. Because proteins are large and complex molecules, it is also important to note that the Setchenow equation need not be satisfied in protein studies if other interactions, such as ionic strength-dependent interactions between the charged protein and salt ions, are present.

Salting out nonpolar molecules and the cavity model

Almost all Hofmeister ions salt out nonpolar molecules from aqueous solution. Fig. 1 shows data for salting out

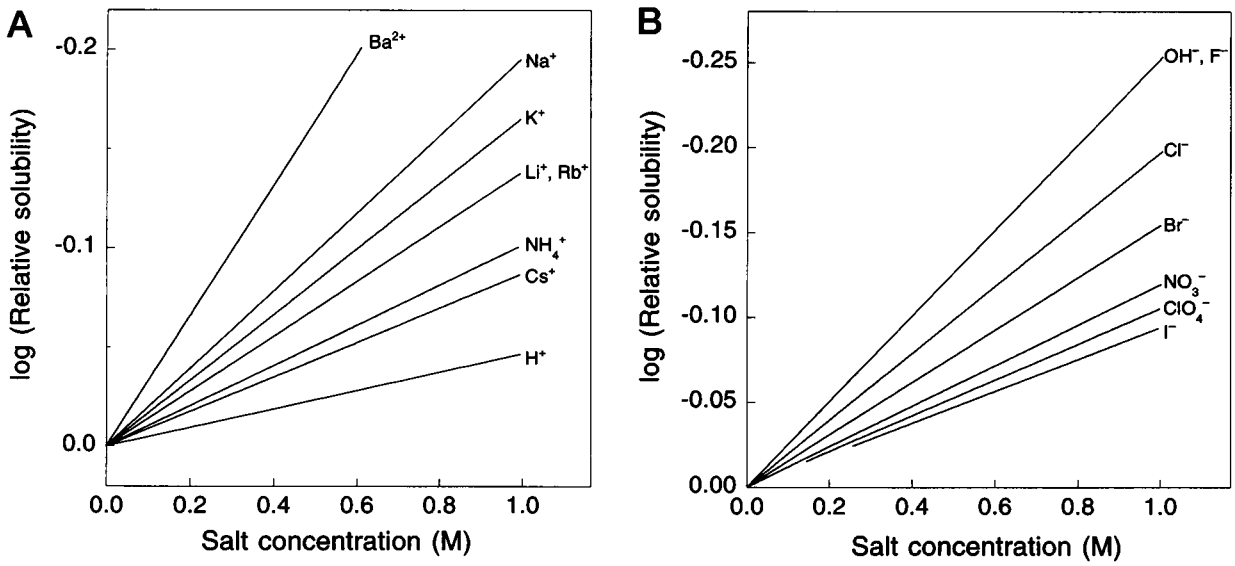

FIGURE 1 Effectiveness of various Hofmeister ions in salting out benzene from aqueous solution. The results are plotted according to the Setchenow equation, which tests if the data fit the weak interaction model. (*A*) Cations (chloride salts); (*B*) Anions (sodium salts). The data are from McDevit and Long (1952); the figure has been redrawn. Relative solubility refers to the ratio of benzene solubilities in the presence of salt (at the concentration indicated) and in the absence of salt.

benzene, reproduced from McDevit and Long (1952). The rank order of effectiveness of the anions in salting out benzene is $SO_4^{2-} > OH^-, F^- > Cl^- > Br^- > NO_3^- > ClO_4^- > I^-$, and the rank order of the cations is $Ba^{2+} > Na^+ > K^+ > Li^+ > Rb^+ > NH_4^+ > Cs^+ > H^+$. The only salts or acids that cause salting in instead, such as $HClO_4^-$ and $(CH_3)_4NBr$, contain large (hydrated) monovalent ions. In general, divalent ions are more effective at salting out than monovalent ions, and ions with small (hydrated) radii are more effective than large ions (see Collins and Washabaugh, 1985). As Fig. 1 shows, the results for benzene uniformly obey the Setchenow equation, and therefore the interaction between benzene and Hofmeister ions obeys the weak interaction model.

Various physical properties of aqueous salt solutions follow the rank order of the Hofmeister series (see Collins and Washabaugh, 1985). Fig. 2 shows the increase in surface tension with salt concentration (reproduced from Jarvis and Scheiman, 1968); the surface tension increment enters into the interpretation of salting-out results via the cavity model. The usefulness of the cavity model in interpreting solubilities and partition coefficients of nonpolar molecules has been known for a long time, at least as far back as Brønsted (1931). The work of making a spherical macroscopic cavity in a liquid is simply the product of the surface area (A) of the cavity times the surface tension (γ) of the liquid:

$$\Delta G = A\gamma. \tag{6}$$

Writing the analogous equation for a microscopic (molecular-size) cavity is a complex problem that is beyond the scope of this discussion. The small size of the solvent (water) is expected to be an important factor contributing to the work of making a microscopic cavity in water, according to Lee (1985) and Madan and Lee (1994). Pratt and Pohorille (1992) argue that the concept of surface tension breaks down for molecular-size cavities. On the other hand, Sharp et al. (1991) argue that the surface tension of the solvent (or the interfacial tension between a liquid hydrocarbon and water) and the accessible surface area of the solute are the basic factors contributing to the work of making a cavity in water, although the macroscopic surface tension must be corrected for the radius of curvature of the microscopic cavity.

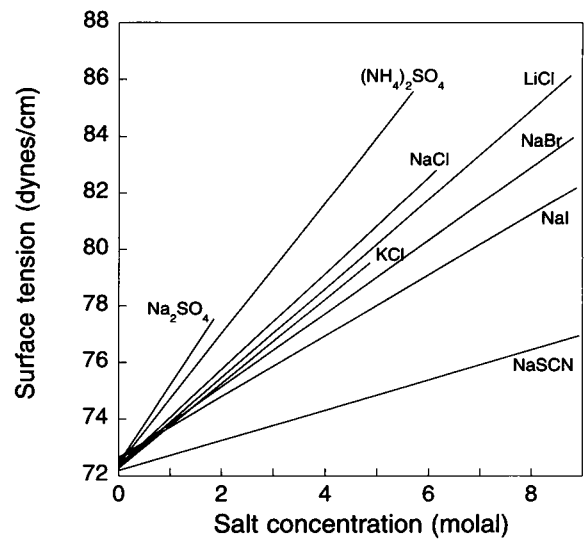

FIGURE 2 Effectiveness of various Hofmeister salts in increasing the surface tension of water at 25°C. Data are from Jarvis and Scheiman (1968); the figure has been redrawn.

Without wishing to enter into this discussion, I point out that the surface of a molecular-size cavity should respond in a manner similar to that of a macroscopic surface to changes in the thermodynamic activity and hydrogen-bonding properties of bulk water caused by adding a Hofmeister salt. Consequently, the changes in macroscopic surface tension produced by a Hofmeister salt should provide a useful guide to what is happening at the surface of a molecular-size cavity (see also Breslow and Guo, 1990). Lin and Timasheff (1996) make a similar argument for the use of surface tension as a guide to understanding how stabilizers and denaturants affect protein stability (see also Arakawa and Timasheff, 1982). They point out that any preferential binding by the protein of the denaturant or stabilizer is also an important factor affecting the results. Thus, the cavity model is used here as a semiquantitative guide.

To interpret the partitioning of a nonpolar solute between water and an immiscible nonpolar liquid, and the effect of salt on the partitioning, Eq. 6 is first written separately for solute i in water (solvent 1) and in the nonpolar liquid (solvent 2). Then the transfer free energy of i is equated to $\Delta\Delta G_i$, the difference between the work of making a cavity in water and in the nonpolar liquid. The result is

$$\Delta\Delta G_i = -RT \ln K_i = A_i(\gamma_1 - \gamma_2), \tag{7}$$

where K_i is the partition coefficient of the solute for equilibration between the two liquids. A corresponding thermodynamic expression for the partition coefficient is obtained by writing Eq. 3 separately for solute i dissolved in water and in the nonpolar liquid, and then setting $\mu_i(1) = \mu_i(2)$.

$$-\ln K_i = \Delta G_i^{\circ}/RT + \beta_{ik}C_k \tag{8}$$

$$\Delta G_i^{\circ} = \mu_i^{\circ}(1) - \mu_i^{\circ}(2) \tag{8a}$$

$$K_i = C_i(1)/C_i(2). \tag{8b}$$

The salt (component k) is assumed not to enter the nonpolar liquid.

To compare Eqs. 7 and 8, the surface tension of the aqueous salt solution is written as a linear function of the salt concentration,

$$\gamma_1 = \gamma_1^{\circ} + \Delta\gamma_k C_k. \tag{9}$$

Then equating Eqs. 7 and 8 gives

$$-RT \ln K_i^{\circ} = A_i(\gamma_1^{\circ} - \gamma_2) \tag{10a}$$

$$RT\beta_{ik} = A_i\Delta\gamma_k, \tag{10b}$$

where K_i° is the partition coefficient of the solute in the absence of salt, $RT\beta_{ik}$ is the product of the surface tension increment ($\Delta\gamma_k$) of the salt, and A_i, the accessible surface area of the solute.

Equation 10b can be applied to data given by Nandi and Robinson (1972a) (see Figs. 3 and 4). They measured salting-out constants of peptides whose amino acid side chains are straight-chain hydrocarbons with 0, 1, 3, or 4 carbon atoms. They found that the salting-out constants are proportional to the number of carbon atoms. The water-accessible surface areas of the corresponding hydrocarbons are only approximately proportional to the number of carbon atoms (Hermann, 1972; Livingstone et al., 1991), and so the conclusion from Fig. 4 is that the salting-out constants of these side chains are approximately proportional to their accessible surface areas. Fig. 5 *A* shows that the salting-out constants are proportional to the surface tension increment of the salt, as expected from Eq. 10b.

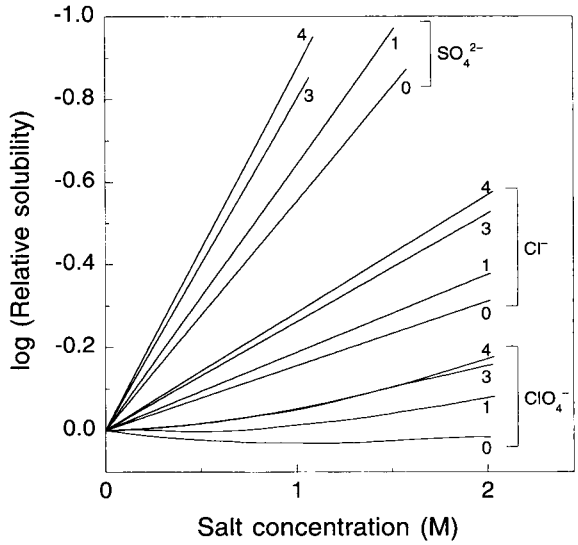

FIGURE 3 Comparison between the effectiveness of Na_2SO_4, NaCl, and $NaClO_4$ in salting out at 25°C blocked amino acids containing straight-chain aliphatic side chains with 0, 1, 3, or 4 carbon atoms. Data are from Nandi and Robinson (1972a); the figure has been redrawn.

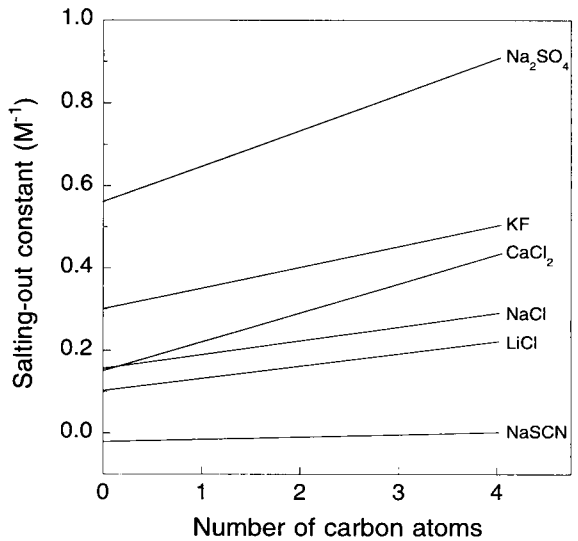

FIGURE 4 Linear dependence of the salting-out constant k_s (see Eq. 1) on the number of side-chain carbon atoms in the blocked amino acids studied by Nandi and Robinson (1972a) (see Fig. 3). The figure has been redrawn.

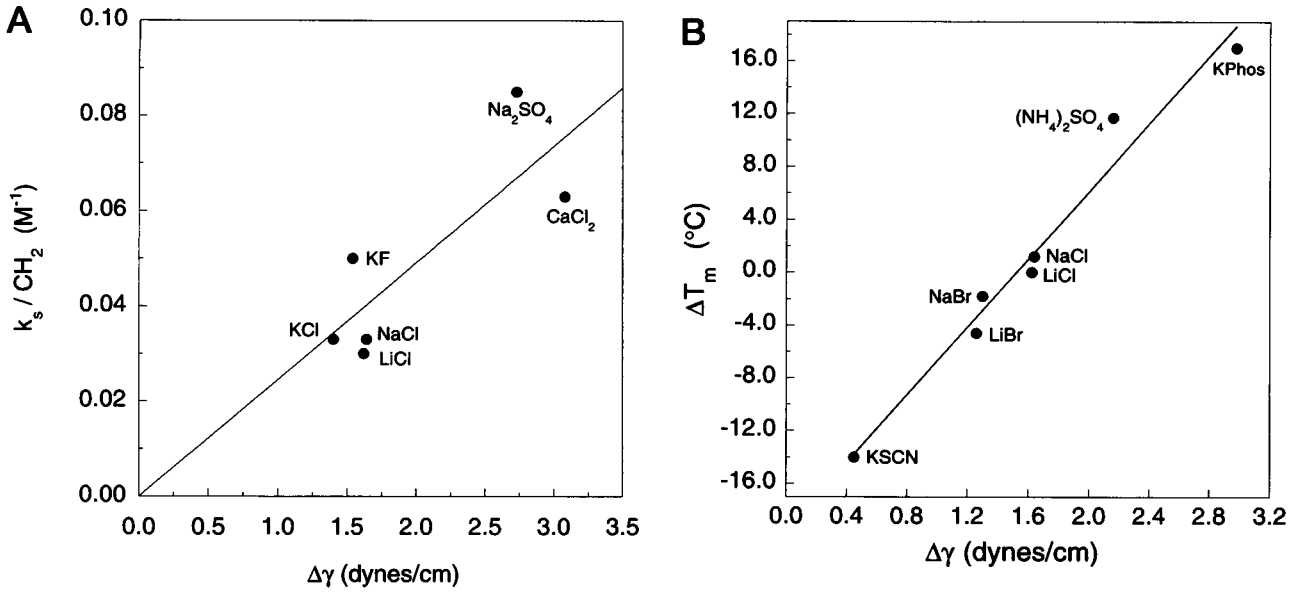

FIGURE 5 (*A*) Salting-out constant, per CH_2 group, versus surface tension increment for various Hofmeister salts. The salting-out constants were measured by Nandi and Robinson (1972a) for amino acid side chains with varying numbers of carbon atoms (see Figs. 3 and 4 here; the values are from their table 4). The surface tension increments refer to 1 molal solution, and the values, except for KF, are taken from the International Critical Tables (Washburn, 1928). (*B*) Change in the melting temperature (T_m) of ribonuclease A produced by various Hofmeister salts (data from von Hippel and Wong, 1964) plotted against the surface tension increment of the salt (1 molal solution). The line is drawn to aid viewing and has no theoretical significance. Note that roughly half the salts are denaturants and half are stabilizers, whereas they should all be stabilizers if the only operative effect is the salting out of nonpolar groups (see Fig. 5 *A*). The data point for $CaCl_2$ is off the line and is not shown.

The agreement between the observed value of $RT\beta_{ik}/\Delta\gamma_k$ and the value of A_i predicted from Eq. 10b is as close as can be expected. The slope of the line in Fig. 5 *A* gives $k_s/\Delta\gamma = 0.025$ erg^{-1} cm^2, which translates to $RT\beta_{ik}/\Delta\gamma = 1.4 \times 10^9$ cm^2 mol^{-1}. The value of k_s in Fig. 5 *A* is given per CH_2 group, and the value of A_i per incremental CH_2 group is 29 Å^2 per molecule (Hermann, 1977; Livingstone et al., 1991) or $A_i = 1.7_5 \times 10^9$ cm^2 mol^{-1}. Larger values of A_i (but less than twofold larger) are obtained if A_i is taken as the mean value for the hydrocarbon, instead of the incremental value given by the difference between two hydrocarbons (see Livingstone et al., 1991). Thus, when the cavity model is used to predict the salting-out constants, the agreement is as good (within a factor of 3) as when it is used to predict the proportionality constant in the linear relation between transfer free energy and accessible surface area. Sharp et al. (1991) discuss the latter issue, and they point out the striking fact that the predicted proportionality constant agrees within a factor of 3 with the observed value before any correction is made for the radius of curvature of the cavity.

Salting in the peptide group

If Hofmeister ions affect protein stability only by salting out nonpolar groups via the cavity model, then all Hofmeister ions should be stabilizers, because they increase the surface tension of water (see Figs. 2 and 5 *A*). In fact, ions such as I^- and SCN^- are strong denaturants (see Fig. 5 *B*; von Hippel and Wong, 1964; Jencks, 1987). The denaturant action of Hofmeister ions like SCN^- results from the fact that they salt in the peptide group, and consequently they interact much more strongly with the unfolded form of a protein than with its native form, and they pull the unfolding reaction. The "null point," which separates protein denaturants from stabilizers, is the result of a balance between these two opposing classes of interaction: salting out nonpolar groups and salting in the peptide group.

Two models have been given for this interaction, and arguments can be made in favor of each model. Nandi and Robinson (1972b) find that it is an ion-specific reaction, that it obeys the weak interaction model and fits the Setchenow equation, and that the order of the salting-in constants is similar to the Hofmeister series (see Table 1). Note, how-

TABLE 1 Salting in the peptide group*

Salt	Salting-in constant (M^{-1})
Na_2SO_4	0.013
KF	0.027
NaCl	0.037
NaBr	0.037
NaTCA#	0.070
$CaCl_2$	0.077
NaSCN	0.077
NaI	0.087
$NaClO_4$	0.097

*Data at 25°C from Nandi and Robinson (1972b). The salting-in constant has a sign opposite that of the salting-out constant k_s given in Eq. 1.
#TCA, trichloroacetate.

ever, that Ca^{2+} strongly salts in the peptide group and SO_4^{2-} does not, whereas both Ca^{2+} and SO_4^{2-} strongly salt out nonpolar groups, as expected from their surface tension increments and use of the cavity model. von Hippel and co-workers (1973) argue that the interaction with the peptide group is a nonspecific ion-dipole interaction and the apparent specificity results from Hofmeister ion interactions with nearby nonpolar groups. They chromatographed Hofmeister ions on a polyacrylamide column, using acrylamide as a model for the peptide backbone, and demonstrated a direct interaction by showing that the elution of ions like I^- and SCN^- is retarded relative to tritiated H_2O as a marker (see Fig. 6). Then Hamabata and von Hippel (1973) varied the number of vicinal methyl groups near the amide group and extrapolated to zero methyl groups, where they found similar intercepts for different Hofmeister ions.

It is difficult to explain the results of Nandi and Robinson (1972b) by the model of von Hippel and co-workers (see Note Added in Proof). Nandi and Robinson varied the number of glycyl peptide groups and found that the salting-in constants of Hofmeister ions are proportional to the number of peptide groups (Fig. 7). Their glycyl peptides contain methyl groups only in the acetyl and ethyl ester blocking groups at the N- and C-termini, and the number of methyl groups is held constant as the number of glycyl peptide groups is varied. The effect of each Hofmeister salt in salting out nonpolar groups appears only in the value of k_s at the y intercept of Fig. 7. Their results fit the Setchenow equation, which indicates that interactions with the peptide group obey the weak interaction model.

Supporting von Hippel's model is older evidence demonstrating the existence of a nonspecific ion-dipole interaction (see Cohn and Edsall, 1943). This interaction is discussed in the following section.

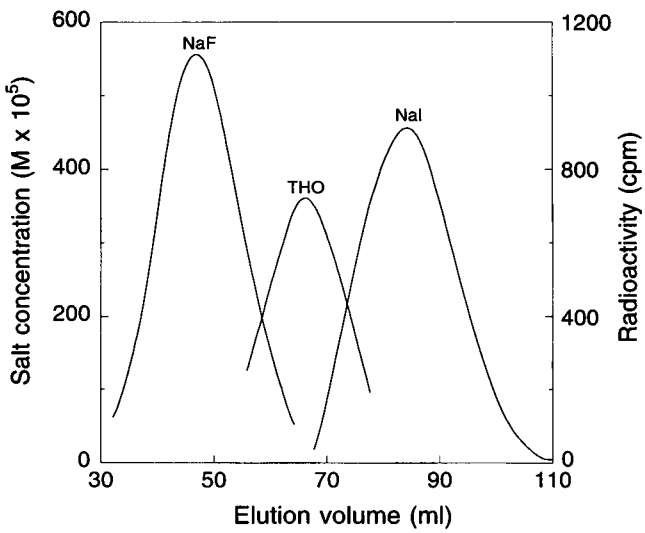

FIGURE 6 Chromatography of two Hofmeister salts on a polyacrylamide column at 25.2°C. Elution volumes relative to tritiated water (TH0) are shown. Data are from von Hippel et al. (1973); the figure has been redrawn.

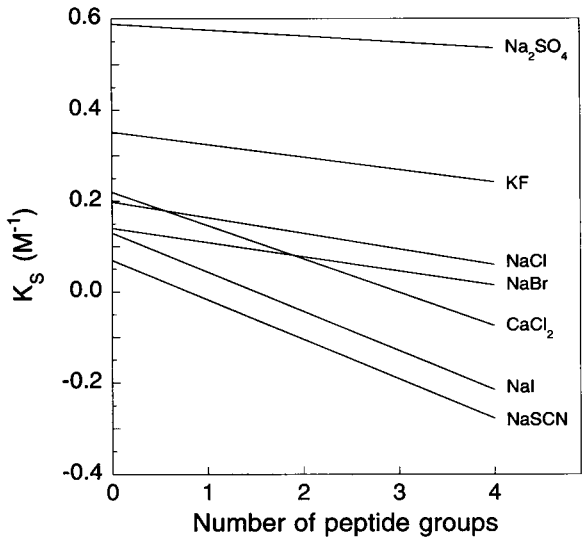

FIGURE 7 The salting-out or salting-in constant of blocked glycyl peptides with one to four peptide groups, at 25°C. Data are from Nandi and Robinson (1972b); the figure has been redrawn. The y intercept shows the salting-out constant of the acetyl and ethyl ester blocking groups. All salts shown produce salting-in of the peptide group, with the change in k_s being proportional to the number of peptide groups.

Salting in and salting out dipolar molecules

When the salting-in or salting-out behavior is measured for molecules that contain both a substantial dipole moment and some nonpolar groups, the results do not obey the Setchenow equation. Instead of finding a linear plot described by a single salting-in or salting-out constant, a curved plot is found. This behavior was noted and analyzed in older work, and a summary is given in Cohn and Edsall (1943). Some results are shown in Fig. 8 for glycine, leucine, cystine, and aspartic acid being salted in or salted out by NaCl (and glycine by KCl). Both cystine and aspartic acid, which have significant dipole moments, are salted in and show evident curvature in the Setchenow plots. These and similar studies led to the conclusion that there is a nonspecific ion-dipole interaction that is dependent on ionic strength. Kirkwood (1943) analyzed the ion-dipole interaction theoretically and succeeded in predicting its magnitude correctly. He found that it is proportional to the first power of ionic strength at low values of the ionic strength.

The peptide group has a significant dipole moment, and it is logical to conclude, as did Hamabata and von Hippel (1973), that Hofmeister ions interact with the peptide group by a nonspecific ion-dipole interaction. This explanation is contradicted, however, by the results of Nandi and Robinson (1972b), who found that the salting-in constants for interaction with the peptide group are specific. They have distinctly different values for SO_4^{2-} and SCN^-, for example, and the linear relation between the salting-in constant and the number of peptide groups indicates that the differences cannot be assigned to Hofmeister interactions with nonpolar groups (see Fig. 7).

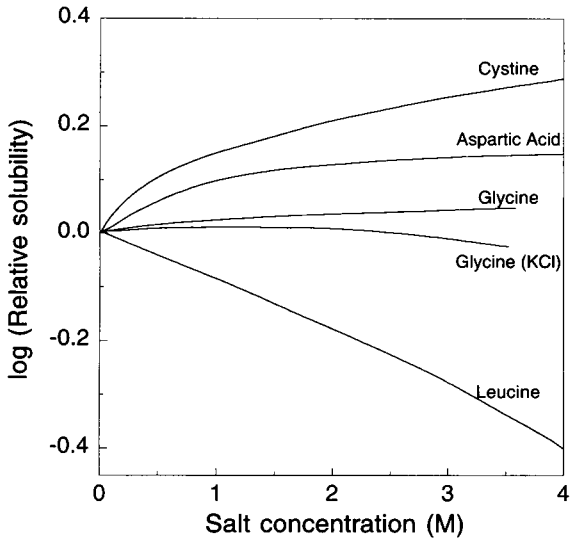

FIGURE 8 Relative solubilities of four amino acids at varying NaCl concentrations; for glycine, the curve for KCl is also shown. The data are from Cohn and Edsall (1943); the figure has been redrawn. Note the deviations shown by cystine and aspartic acid from the linear behavior predicted by the Setchenow equation.

A nonspecific salting-out (helix-stabilizing) interaction was observed at ionic strength values below 0.15 M for three Hofmeister salts interacting with an uncharged peptide helix (Scholtz et al., 1991). Effects of the Hofmeister interactions could be subtracted by measuring k_s at high values of the ionic strength (above 1 M). The helix-stabilizing effect was ascribed to screening of the field of the helix dipole. The study illustrates the diversity of effects encountered in studies of ion-protein interactions.

CONCLUDING REMARKS

Ions interact with proteins in a variety of ways. Often these interactions are specific to the protein and to the particular conditions employed (pH, temperature). Many interesting examples have been analyzed by Serge Timasheff and his co-workers. The Hofmeister ion interactions are unusual because they show a similar pattern, not only with different proteins but even with DNA and collagen (von Hippel and Wong, 1964). The reason for the similar pattern can be understood when the cavity model is used to interpret the salting-out of nonpolar groups, but the validity of the cavity model at the molecular level is controversial and seems likely to remain so. A different mechanism is needed to interpret the interactions of Hofmeister ions with the peptide group, because these interactions are opposite in sign. The question of whether the peptide group interactions are ion-specific remains controversial. The fundamental question of how Hofmeister ions affect the structure and hydrogen-bonding properties of water has not been discussed here.

Note added in proof—Dr. P. H. von Hippel points out that the glycine peptides studied by Nandi and Robinson (1972b) contain a methylene group in the peptide backbone of each glycyl residue. Thus, the ion-specific interactions with the glycyl residue (see Fig. 7, this article) may result from a nonspecific salting-in interaction with the peptide group plus an ion-specific salting-out interaction with the methylene group.

The following observations indicate, however, that this model cannot explain the contrasting behavior of Na_2SO_4 and $CaCl_2$ in interacting either with the glycyl residue (Nandi and Robinson, 1972b; see Fig. 7, this article) or with a polyacrylamide column (von Hippel et al., 1973). $CaCl_2$ and Na_2SO_4 salt-out the methylene group in a similar way, as expected from the fact that they have similar surface tension increments (see Fig. 5*A*). Thus the model of von Hippel et al. (1973) predicts that they should behave in a similar way either as protein denaturants or in interacting with the glycyl residue or polyacrylamide. Na_2SO_4 is, however, strongly stabilizing while $CaCl_2$ is a strong denaturant: see data for Ca^{2+} and SO_4^{2-} acting on RNase A and on collagen (von Hippel and Wong, 1964) and on an alanine-based peptide helix (Scholtz et al., 1991).

It is a pleasure to dedicate this paper to Serge Timasheff, whose work has done so much to shape the concepts in this field.

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