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Biophysical Chemistry 105 (2003) 517–532

Biophysical Chemistry

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Preferential interactions in aqueous solutions of urea and KCl☆

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Received 2 December 2002; accepted 16 December 2002

Abstract

A quantitative characterization of the thermodynamic effects due to interactions of salt ions and urea in aqueous solution is needed for rigorous analyses of the effects of changing urea concentration on biopolymer processes in solutions that also contain salt. Therefore, we investigate preferential interactions in aqueous solutions containing KCl and urea by using vapor pressure osmometry (VPO) to measure osmolality as a function of the molality of urea (component 3) over the range $0.09 \leqslant m_3 \leqslant 1.65$ m at two fixed molalities of KCl (component 2) ($m_2 = 0.212$ and 0.427 m). With this experimental input and corresponding VPO measurements on solutions that contain only urea or KCl, we evaluate approximately the chemical potential derivative $\mu_{23} = (\partial\mu_{\mathrm{KCl}}/\partial m_{\mathrm{urea}})_{T,P,m_{\mathrm{KCl}}} = (\partial\mu_{\mathrm{urea}}/\partial m_{\mathrm{KCl}})_{T,P,m_{\mathrm{urea}}} = \mu_{32}$ and hence the preferential interaction coefficients $\Gamma\mu_3$ and $\Gamma\mu_1,\mu_3$. These results show that for water–KCl–urea solutions neither of these coefficients is determined primarily by contributions from thermodynamic nonideality to μ_{23}. In aqueous solutions containing a biopolymer and a small solute, the contribution of ideal mixing entropy to μ_{23} is negligible in comparison with the experimental uncertainty, whereas in KCl–urea solutions the contribution due to ideal mixing entropy accounts for at least half of the magnitude of μ_{23}. For comparison, we analyze literature data for NaCl–urea interactions and find again that nonideality makes a smaller contribution to μ_{23} than does ideal mixing entropy. In contrast, for aqueous solutions of urea and the protein bovine serum albumin, the experimentally determined contribution of nonideality to μ_{23} exceeds the contribution of ideal mixing by a factor of $\sim 2\times 10^2$.

Keywords: Preferential interactions; Vapor pressure osmometry; Thermodynamic nonideality; Solution thermodynamics

1. Introduction

The concentrations (or thermodynamic activities) of comparatively small solute molecules or ions constitute important variables for the regulation of processes involving biopolymers. The thermodynamics of many biopolymer processes, including protein folding and protein-DNA binding, are strongly perturbed by solutes such as urea, glycerol, sugars, glycine betaine and/or salts like KCl that apparently do not act as direct stoichiometric participants (i.e. do not bind as ligands to sites on any reactant or product of the process). Preferential interactions between a perturbing sol-

☆ Dedicated to Walter Kauzmann, whose pioneering studies of protein stability demonstrated the power of rigorous thermodynamic analysis of biopolymer processes.

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doi:10.1016/S0301-4622(03)00112-1

ute and a biopolymer give rise to a solute-concentration dependence of the biopolymer activity coefficient and therefore cause the observed equilibrium constant (i.e. the stoichiometric quotient of product and reactant concentrations at equilibrium) for a biopolymer process to depend on the concentration of the perturbing solute. The model-independent expressions needed for rigorous thermodynamic analyses of such effects were presented originally for a single uncharged perturbing solute by Wyman [1] and derived more generally for systems where the perturbing solute is either uncharged or a strong electrolyte [2,3].

In many cases, solute–protein interactions and solute effects on protein processes can be interpreted in terms of a two-domain (local-bulk) model [3–7]. For example, an analysis based on this model shows that at a specified concentration of a particular solute, the magnitude of the preferential interaction coefficient is directly proportional to the water accessible protein surface area (in a homologous series of proteins having comparable surface compositions). Hence, solute-concentration-dependent effects on processes involving proteins can be analyzed in favorable cases to obtain structural information, specifically the change in surface area that accompanies a process such as protein unfolding [6–8].

Both in vivo and in vitro, many important solute effects on biopolymer processes occur in systems containing more than one perturbing solute. For example, effects of osmolytes and other uncharged solutes on processes that involve a nucleic acid must be studied in the presence of salt (e.g. NaCl, KCl). Consequently, for comprehensive thermodynamic analyses of such effects, interactions involving only the small molecules of the perturbing solute and salt ions must be considered in addition to interactions of the perturbing solute component(s) with the biopolymers that participate in the stoichiometric process. Recently, we (Hong et al., in preparation) have investigated the effects of varying the concentration of urea on a specific protein-DNA binding process in solutions that also contain KCl at two fixed concentrations. Rigorous thermodynamic analysis of such processes (studied under typical experimental conditions) requires a type of coefficient that characterizes the interactions of salt ions and urea in a three-component solution, in addition to the coefficients that characterize the preferential interactions of urea with each stoichiometric participant in the process.

A primary objective of the present study is to quantify the salt–urea preferential interaction coefficients that are required for analyses of the effects of changing urea concentration on protein-DNA binding in the presence of KCl (Hong et al., in preparation). We also introduce in this paper a new way of approximating the coefficients that characterize the preferential interactions of two solute components with each other in aqueous solution. Quantitative information about the preferential interactions of salt ions and urea should also prove useful as a point of reference for interpretations of the interactions of these ions with amide groups on proteins, and of the interactions of urea with charged groups on biopolymers.

2. Thermodynamic fundamentals

As defined for a three-component solution, preferential interaction coefficients express the functional linkage between the molalities of the two solutes when three thermodynamic functions are fixed: T, and two chosen from P, μ_1 and μ_3 [9]. In accordance with the usual convention, the numerical labels 1, 2 and 3 denote, respectively, solvent water and the two solute components: KCl (component 2) and urea (component 3). The three coefficients of interest here are distinguished by the subscript(s) on Γ that denote the chemical potential(s) held fixed:

$$\Gamma\mu_1 \equiv \left(\partial m_3/\partial m_2\right)_{T,P,\mu_1} \tag{1a}$$

$$\Gamma\mu_3 \equiv \left(\partial m_3/\partial m_2\right)_{T,P,\mu_3} \tag{1b}$$

$$\Gamma\mu_1,\mu_3 \equiv \left(\partial m_3/\partial m_2\right)_{T,\mu_1,\mu_3} \tag{1c}$$

Thermodynamic relationships linking these preferential interaction coefficients have been derived [10,11], and their distinguishing characteristics discussed [5]. For many types of solutes under most conditions, $\Gamma\mu_1$ is the coefficient most accessible to accurate experimental determination, via

either vapor pressure osmometry (VPO) or isopiestic distillation (ID). However, $\Gamma\mu_3$ has the most direct utility for rigorous thermodynamic analyses of solute effects on biopolymer processes [2], and $\Gamma\mu_1,\mu_3$ is most directly interpretable using the local-bulk domain model of solute–biopolymer interactions [3–5].

In this paper, we analyze preferential interactions in a three-component system where the two biologically significant solutes are similar in molecular size. For this purpose we introduce a new way of calculating $\Gamma\mu_3$, based on an approximation that originally was devised to calculate the activity coefficient of each solute in a three-component system by utilizing the Gibbs–Duhem equation to analyze input obtained from ID measurements [12,13]. Previously, two other approximate expressions (described in detail in Ref. [10]) have been applied to calculate $\Gamma\mu_3$ and hence $\Gamma\mu_1,\mu_3$ by analyzing VPO measurements that characterize preferential interactions in three-component solutions containing the protein bovine serum albumin (BSA) and any of a wide variety of denaturants and osmolytes [5,7].

As in applications of each of the approximate expressions used previously, the approach introduced here to calculate $\Gamma\mu_3$ is based on an indirect way of approximating the cross-partial derivative $\mu_{23}=\mu_{32}$, which expresses the dependence of the chemical potential of either solute component on the molality of the other. The conventional double subscript notation μ_{ij} denotes the partial derivative $(\partial\mu_i/\partial m_j)_{T,P,\{m_{j'\neq j}\}}$ for $i=1, 2, 3$ and $j=2, 3$, where the subscript $\{m_{j'\neq j}\}$ indicates that each molality other than m_j is fixed. By standard mathematical manipulations [9], $\Gamma\mu_3$ can be represented, in a form exactly equivalent to the definition Eq. (1b), as the following quotient of derivatives of chemical potentials:

$$\Gamma\mu_3=-\mu_{23}/\mu_{33}=m_3\mu_{23}/(m_1^{\bullet}\mu_{13}+m_2\mu_{23}) \qquad (2)$$

The second equality in Eq. (2), which follows from a linkage expression based on the Gibbs–Duhem equation [10], shows that, for a given specification of m_2 and m_3, the derivative of the chemical potential of solute component 3 with respect to its own molality (μ_{33}) is related to μ_{13}, the corresponding derivative of the solvent (component 1) chemical potential, multiplied by $m_1^{\bullet}$, the fixed 'molality' of solvent water (55.5 mol kg^{-1}). By definition osmolality, which is measured directly by a water vapor pressure osmometer, is related to the activity of solvent water (a_1) by $\mathrm{Osm}\equiv-m_1^{\bullet}\ln a_1$. Hence, the derivative of Osm with respect to m_3 is simply proportional to the corresponding derivative of μ_1, the chemical potential of solvent water, and for applications of Eq. (2), VPO measurements of Osm as a function of urea molality can be fitted to determine the derivative μ_{13} without approximation.

In addition to experimentally determined values of μ_{13}, calculations of $\Gamma\mu_3$ using Eq. (2) require only μ_{23}. This derivative can be estimated by relating it to the 'osmolality increment' defined as:

$$\Delta\mathrm{Osm}\equiv\mathrm{Osm}(m_2,m_3)-\mathrm{Osm}(m_2)-\mathrm{Osm}(m_3) \qquad (3)$$

The terms $\mathrm{Osm}(m_2)$ and $\mathrm{Osm}(m_3)$ denote the osmolalities measured in each of the two-component solutions that correspond to the three-component solution for which $\mathrm{Osm}(m_2,\ m_3)$ is measured. The thermodynamic function ΔOsm differs from zero both because of interactions between the two solute components and because of contributions due to ideal mixing entropy, which have no dependence on the molecular size or any chemical characteristics of either solute component. We therefore define an excess function $\Delta\mathrm{Osm}^{\mathrm{ex}}$, which depends entirely on thermodynamic consequences of solute–solute preferential interactions, as:

$$\Delta\mathrm{Osm}^{\mathrm{ex}}\equiv\Delta\mathrm{Osm}-\Delta\mathrm{Osm}^{\mathrm{mix}} \qquad (4)$$

In Eq. (4), $\Delta\mathrm{Osm}^{\mathrm{mix}}$ is defined by analogy to Eq. (3) using expressions for the contributions of ideal mixing entropy to osmolality in each of the three- and two-component solutions.

For the system of primary interest here, an aqueous solution containing a 1:1 salt component 2 (such as KCl) and a nonelectrolyte component 3 (urea):

$$\begin{aligned}\mathrm{Osm}^{\mathrm{mix}}(m_2,m_3)&=-m_1^{\bullet}\ln x_1\\&=-m_1^{\bullet}\ln\left(m_1^{\bullet}/(m_1^{\bullet}+2m_2+m_3)\right)\end{aligned} \qquad (5)$$

where x_1 is the mole fraction of the solvent. Corresponding expressions for $Osm^{mix}(m_2)$ and $Osm^{mix}(m_3)$ are obtained from Eq. (5) by setting the appropriate solute molality equal to zero. Because, under the conditions of interest here, $2m_2$ and m_3 are always small compared with $m_1^\bullet$, each of the three terms that comprise ΔOsm^{mix} depends only slightly on either m_2 or m_3 and the net concentration dependence of ΔOsm^{mix} is even smaller than those of the individual terms.

The original analysis of Robinson and Stokes [12] is readily modified to relate ΔOsm^{ex} approximately to μ_{23}^{ex}, the derivative with respect to m_3 of the excess chemical potential $\mu_2^{ex}$of component 2:

$$\mu_{23}^{ex} = RT\left(\frac{\partial \ln\gamma_2}{\partial m_3}\right)_{T,P,m_2} \cong \frac{RT\Delta Osm^{ex}}{m_2 m_3} \tag{6}$$

In Eq. (6), $\mu_2^{ex} \equiv RT\ln\gamma_2$, where γ_2 (equal to the square of the mean ionic activity coefficient $\gamma_\pm$) is defined here as the mole fraction-scale activity coefficient that expresses the nonideality of the 1:1 electrolyte solute component (2) relative to the conventional 'ideal dilute' solution reference state, in which the univalent cations and anions—and any other solute in the system—interact only with solvent and solvent molecules interact only with each other. By analogy to Eq. (6), μ_{23}^{mix}, the contribution to μ_{23} arising from ideal mixing entropy, is related approximately to ΔOsm^{mix} [11]:

$$\mu_{23}^{mix} = 2RT\left(\frac{\partial \ln x_i}{\partial m_3}\right)_{T,P,m_2} = -\frac{2RT}{m_1^\bullet + 2m_2 + m_3} \cong \frac{RT\Delta Osm^{mix}}{m_2 m_3} \tag{7}$$

where x_i is the mole fraction of the univalent cation or anion. By definition, the sum of μ_{23}^{mix} and μ_{23}^{ex} must equal μ_{23}:

$$\mu_{23} \equiv \mu_{23}^{mix} + \mu_{23}^{ex} \tag{8}$$

For low molal concentrations of a polymeric solute (2) and an excess of a low molecular weight solute (3), the magnitudes of μ_{23} and ΔOsm typically are dominated by contributions from nonideality due to preferential interactions, (and the experimental uncertainty in μ_{23} greatly exceeds μ_{23}^{mix}, as discussed subsequently). However, for the systems and conditions of interest here, the corresponding contributions due to ideal mixing entropy cannot be neglected a priori.

Values of the preferential interaction coefficient $\Gamma\mu_1,\mu_3$ generally can be quantified by dialysis in cases where the solutes differ substantially in size. Even for systems in which both (or neither) of the solutes would diffuse across a dialysis membrane, $\Gamma\mu_1,\mu_3$ remains a well-defined thermodynamic function. This coefficient can be calculated with input from osmometric and density measurements at constant T and P (i.e. without dialysis) by the relationship [11]:

$$\Gamma\mu_1,\mu_3 = (1+Q_v)^{-1}(\Gamma\mu_3 + Q_v\Gamma\mu_1) \tag{9a}$$

Here Q_v is defined:

$$Q_v \equiv m_3\bar{V}_3\left(1 - m_2\Gamma\mu_3/m_3\right)/\left(m_1^\bullet\bar{V}_1\right) \tag{9b}$$

Under typical conditions of interest, the term $m_2\Gamma\mu_3/m_3$ in Eq. (9b) makes at most a minor contribution to Q_v, which depends primarily on the ratio of the partial molar volumes $\bar{V}_3$ and $\bar{V}_1$ of the components for which the chemical potentials are held fixed. Eqs. (9a) and (9b) have been applied to analyze VPO data in solutions containing BSA and a small solute of various types [5].

For the solute–protein systems that have been investigated so far [5,7], $0 < Q_v \ll 1$, and the term $|Q_v\Gamma\mu_1| \ll |\Gamma\mu_3|$. Consequently, $\Gamma\mu_1,\mu_3 \cong \Gamma\mu_3$, even though in all of those systems $\Gamma\mu_1$ differs significantly from both $\Gamma\mu_3$ and $\Gamma\mu_1,\mu_3$. In contrast, detectable differences between $\Gamma\mu_3$ and $\Gamma\mu_1,\mu_3$ have been determined by application of Eqs. (9a) and (9b) to analyze ID data for aqueous solutions containing urea and NaCl (as demonstrated in Fig. 2 of Ref. [11]). Nevertheless, for this system over the entire ranges of m_2 and m_3 the difference between $\Gamma\mu_3$ and $\Gamma\mu_1,\mu_3$ is considerably smaller in magnitude than the corresponding differences between either $\Gamma\mu_3$ or $\Gamma\mu_1,\mu_3$ and $\Gamma\mu_1$.

3. Experimental procedure and methods

KCl (certified A.C.S, FW 74.56, 99.5% pure) was obtained from Fisher Scientific. Urea was obtained from Fluka BioChemika (>99.5% pure, FW 60.06) and from Life Technologies (>99.5% pure, subsequently specified as ⩾98%). According to Life Technologies, their estimate of purity was revised only to take more realistic account of the uncertainty (2%) in the analytic procedure used to assess purity, and does not, for example, result from any alteration in the procedure used to produce the chemical. For samples made up from any of the three sources of urea, we find good reproducibility of the VPO measurements at each concentration of each solute in the ranges investigated.

All of the volumes measured by pipette for our sample preparations were calibrated gravimetrically. At 0.212 m and 0.427 m KCl, salt concentrations which fall within the typical range over which specific protein-DNA binding is studied in vitro, a 1 ml volume of a urea stock solution was prepared gravimetrically. (For urea concentrations below 0.25 m, the stock solution was prepared volumetrically in 100 ml.) For a 4.000 ± 0.002 M KCl solution made up volumetrically in 100 ml, the density was measured by a vibrating-tube density meter (DMA 5000, Anton Paar) to obtain the 'weight concentration' (grams of KCl and water in a known solution volume). Knowledge of the weight concentration is required for accurate specifications of molalities in sample preparations. Predetermined amounts of the urea stock solution and 4.000 M KCl were combined gravimetrically to obtain solutions at either 0.212 ± 0.002 or 0.427 ± 0.002 m KCl. This method of preparation permits direct evaluations of $(\partial \mathrm{Osm}/\partial m_3)_{T,P,\mathrm{m}_2}$, hence of μ_{13} in Eq. (2), by fixing m_2, rather than the corresponding molarity, which is operationally somewhat simpler to hold constant. However, the latter approach requires converting $(\partial \mathrm{Osm}/\partial m_3)_{T,P,C_2}$ to $(\partial \mathrm{Osm}/\partial m_3)_{T,P,\mathrm{m}_2}$ by introducing partial molar volumes (Eq. (17) in Ref. [5]), with their concomitant contributions to the propagated error.

Relevant background on VPO was presented previously [14]. In the present study, two osmometers, Wescor 5500 and VAPRO 5520 (Logan, UT) were used, because VAPRO 5520 does not operate reliably when the ambient relative humidity exceeds 42% (whereas Wescor 5500 does) but has a more accurate procedure for standard calibration than does Wescor 5500. No significant differences in readings of Osm between the two osmometers were found above 40 mOsm for systems used in this study, although they have different working temperatures (37 °C for Wescor 5500 and for VAPRO 5520 ambient, but fixed, in the range 23–25 °C). The procedures used to clean the thermocouple and calibrate the instrument were the same as those described in detail in Ref. [5]. During a series of osmometer readings, re-calibration was generally performed less than 1 h after the preceding calibration. Three aliquots from each sample were measured in immediate sequence.

According to the Wescor manual [15], the standard NaCl solutions supplied by Wescor for calibration are made up on the basis of reference data for aqueous NaCl solutions taken from the *Handbook of Physics and Chemistry*, CRC press (e.g. 59th edition [16]). After completion of most of the VPO experiments reported here, we discovered that these CRC data are not in agreement with more recent literature values, summarized and analyzed in a study [17] which utilized Pitzer's equation [18,19]. All osmometer readings that initially had been calibrated using Wescor standards were corrected using this fitting equation [17], whereby the osmolalities of the standards (specified as 0.29, 1.000 Osm) were revised to 0.2905 and 1.015 Osm, respectively. The 1 Osm standard was used to adjust the slope of the response curve (a presumably linear relationship between the voltage change and the osmolality); the 0.29 Osm standard was used for the offset control. Revision of the osmolalities pertaining to these standard solutions therefore scales all original VPO readings by a factor of 1.015. Over the concentration range investigated here, we find that this correction is significant as compared with experimental uncertainties in the osmolality measurements, whereas the additive offset correction ($+0.0005$) is insignificant.

Experimental uncertainty in osmometer readings arises from: (i) instrument fluctuations for any

sample in immediate repeated readings; (ii) the offset control; and (iii) the gain control. Based on the working principle of the osmometer, the formula we used to estimate the uncertainty in each osmometer reading is:

$$\sigma_i^2 = \sigma_o^2 + \sigma_f^2 + \sigma_g^2\left(\mathrm{Osm}_i/1.015\right)^2 \tag{10}$$

In Eq. (10), σ_o is the error in calibrating the VPO offset control at 0.2905 Osm (generally less than 0.002 Osm), σ_f is the instrument fluctuation obtained from a standard deviation of triplicate readings of samples in this study (generally less than 0.003 Osm), σ_g is the error in calibrating the VPO gain control (generally less than 0.002), Osm_i is the corrected calibrated osmometer reading (in osmolal units of mol kg^{-1}) and 1.015 is the osmolality of the standard solution used to calibrate gain control. (Compared with these contributions any uncertainty associated with the concentration of NaCl in the standard calibrating solution is negligible.)

For utilization of Eqs. (2)–(8), osmolalities and the chemical potential derivative μ_{13}, are the only experimental input needed to calculate $\Gamma\mu_3$. Values of μ_{13} were obtained (related to derivatives of Osm with respect to m_3) by differentiation of the (quadratic) analytic functional form that provides the best fittings to the experimental plots of Osm vs. m_3. Our calculations of $\Gamma\mu_1$, which is needed to evaluate $\Gamma\mu_1$,μ_3 by Eq. (9a), are based on the 'iso-osmolal method' [5] in which the derivative $(\partial m_3/\partial m_2)_{\mu_1,T,P}$ is approximated by the corresponding quotient of differences:

$$\Gamma\mu_1 \cong (m_3^{(a)} - m_3^{(b)})/(m_2^{(a)} - m_2^{(b)}) \tag{11}$$

where (a) and (b) denote two isoosmolal solutions having different concentrations of components 2 and 3. Thus, at each m_3 over the entire range of osmolalities measured, $\Gamma\mu_1$ at 0.212 m KCl was approximated by using the difference in m_3 between isoosmolal solutions containing 0.427 and 0 m KCl (determined by interpolations on best-fitted curves of Osm vs. m_3). At 0.427 m KCl, $\Gamma\mu_1$ was approximated with Eq. (11) by using the difference in m_3 between isoosmolal pairs of urea solutions at 0.427 and 0.212 m KCl.

Because polynomial functions were found sufficient to fit all the data acquired in this study, multiple linear regressions [20] were performed. Based on the matrix solution for application of the method of maximum likelihood to polynomial fitting [20], analytic programs were written and used in this study. As compared with applications to polynomial functions of the non-linear fitting program given by Sigma Plot (from SPSS Inc.), the analytic program used here gave more realistic (lower) uncertainties in the fitting parameters. Error propagation was performed by the method explained in Ref. [20] (Eq. 3.13). The covariance of correlated parameters was included in error propagation because fitting parameters for polynomial functions, generally, are highly correlated.

Our approach to error propagation is illustrated by the following two examples. In accordance with Eq. 3.13 in Ref. [20], the variance of μ_{23}^{ex} is:

$$\sigma^2_{(\mu_{23}{}^{ex})} = \sigma^2_{\Delta\mathrm{Osm}}\left(\frac{RT}{m_2 m_3}\right)^2 \tag{12}$$

The correlation between the variance of m_2 in Eq. (12) and of ΔOsm is negligible, as are the variance of m_2 and m_3 in Eq. (12) and of $\Delta\mathrm{Osm}^{\mathrm{mix}}$. Based on Eq. (3) and the error propagation formula, the variance on ΔOsm is calculated by

$$\sigma^2_{\Delta\mathrm{Osm}} = \sigma^2_{\mathrm{Osm}(m_2,m_3)} + \sigma^2_{\mathrm{Osm}(m_2)} + \sigma^2_{\mathrm{Osm}(m_3)} \tag{13}$$

By Eq. (13), the variance is propagated from the standard error in the fitting parameters for $\mathrm{Osm}(m_2, m_3)$ and $\mathrm{Osm}(m_3)$, and for $\mathrm{Osm}(m_2)$, from the error in m_2. (For $\mathrm{Osm}(m_2)$, unlike $\mathrm{Osm}(m_3)$, an independent fitting function, based on independent KCl titration or literature data [21], was used.) The fitting functions used for $\mathrm{Osm}(m_2, m_3)$ and $\mathrm{Osm}(m_3)$ each have the form:

$$\mathrm{Osm} = \alpha_1 + \alpha_2 m_3 + \alpha_3 m_3^2 \tag{14}$$

Therefore, the corresponding error propagation is

$$\sigma^2_{\mathrm{Osm}} = \sigma^2_{\alpha_1} + \sigma^2_{\alpha_2} m_3^2 + \sigma^2_{\alpha_3} m_3^4 + 2\sigma^2_{\alpha_1\alpha_2} m_3 + 2\sigma^2_{\alpha_1\alpha_3} m_3^2 + 2\sigma^2_{\alpha_2\alpha_3} m_3^3 \tag{15}$$

Osmolalities of two component KCl solutions (Osm(m_2)) at 0.212 m and 0.427 m KCl were calculated from the most recent parameterization [21] of the Pitzer equation for 1:1 salts [18,19]. Because no errors in the fitting parameters were reported, the variance of Osm(m_2) is calculated by propagating the error in m_2 (reduced by a factor of $1/N^{1/2}$ where N is the number of independent data sets at the same m_2 used in the global fitting).

Under the assumption that μ_{23}^{ex} has no significant dependence on m_2 or m_3, the error weighted average value of μ_{23}^{ex} over all m_2 and m_3 examined is calculated from:

$$\overline{\mu_{23}^{ex}} = \frac{\sum\left(\frac{(\mu_{23}^{ex})_i}{\sigma_i^2}\right)}{\sum\left(\frac{1}{\sigma_i^2}\right)} \tag{16}$$

here $(\mu_{23}^{ex})_i$ and σ_i designate, respectively, the value of μ_{23}^{ex} and its standard error for the ith data point. The corresponding uncertainty of the mean $\overline{\mu_{23}^{ex}}$ is calculated from:

$$\sigma_\mu^2 = \frac{1}{\sum 1/\sigma_i^2} \tag{17}$$

4. Results and analysis

For our quantitative investigation of the interactions of urea and the ions K^+ and Cl^- in aqueous solution, VPO was performed to monitor changes in osmolality during urea 'titrations' of samples in which the molality of KCl was held constant at 0, 0.212 or 0.427 m. Fig. 1 presents five data sets for Osm as a function of urea concentration at 0.427 m KCl, three data sets at 0.212 m KCl, and three data sets for urea in the absence of KCl. (In addition, four VPO data sets were collected for KCl solutions containing no urea; cf. Fig. 2 below.) The plots demonstrate good reproducibility of the osmolality measured at each KCl concentration. The three curves are almost parallel; therefore, Eqs. (3)–(6) imply that contributions of urea and KCl to Osm are nearly additive and that the net effect on Osm due to interactions of urea and KCl is small.

In view of the good reproducibility of these VPO data, global fittings of Osm with respect to m_3 were performed to obtain the input needed to calculate $\Gamma\mu_3$. Global fitting was applied only to data points falling within a range where all titration series were represented: 0.17–1.65, 0.3–1.56 and 0.09–1.6 m for the 0, 0.212 and 0.427 m KCl solution data sets, respectively. Under the conditions investigated, the quadratic functional form was found sufficient to describe the dependence of Osm on m_3 for aqueous urea in the presence or absence of KCl, as confirmed by the F_R-test and the multiple-correlation coefficient R^2 [20]. Furthermore, the F-test for an additional (i.e. cubic) term [20] in each case showed that a quadratic function provides the best fitting. In accordance with Eqs. (14) and (15), the average error for Osm_i, calculated by using fitting functions and corresponding errors in their fitting parameters (α_1, α_2, α_3), is 0.001 Osm. Slopes obtained by differentiating the best-fitted functional form of Osm(m_2, m_3) were used to evaluate μ_{13} as input for Eq. (2).

In Fig. 2, our osmolality measurements for two-component aqueous urea solutions are compared with those reported by Scatchard et al. [22] and by Bower and Robinson [13], which are consistent with each other over this concentration range. Fig. 2 also reports measurements of Osm for the two-component aqueous KCl solutions in this study, compared with osmolalities calculated from a recent application [21] of the Pitzer equation [18,19] to extensive literature data. The overall agreement for both urea and KCl is good. Fitting parameters for all VPO data sets are listed in Table 1.

On the basis of Eq. (6), experimental determinations of ΔOsm^{ex} were used to determine values of μ_{23}^{ex}. Quadratic functions fitted to VPO measurements (given in Table 1) were used to calculate Osm(m_2, m_3), Osm(m_3) and, by analytic differentiation, the derivative $(\partial Osm/\partial m_3)_{T,P,m_2}$. In our analysis, calculations using the Pitzer equation [21] were performed to obtain the values of Osm(m_2): 0.387 ± 0.002 at 0.212 m KCl and 0.770 ± 0.002 at 0.427 m KCl. These values agree with our VPO

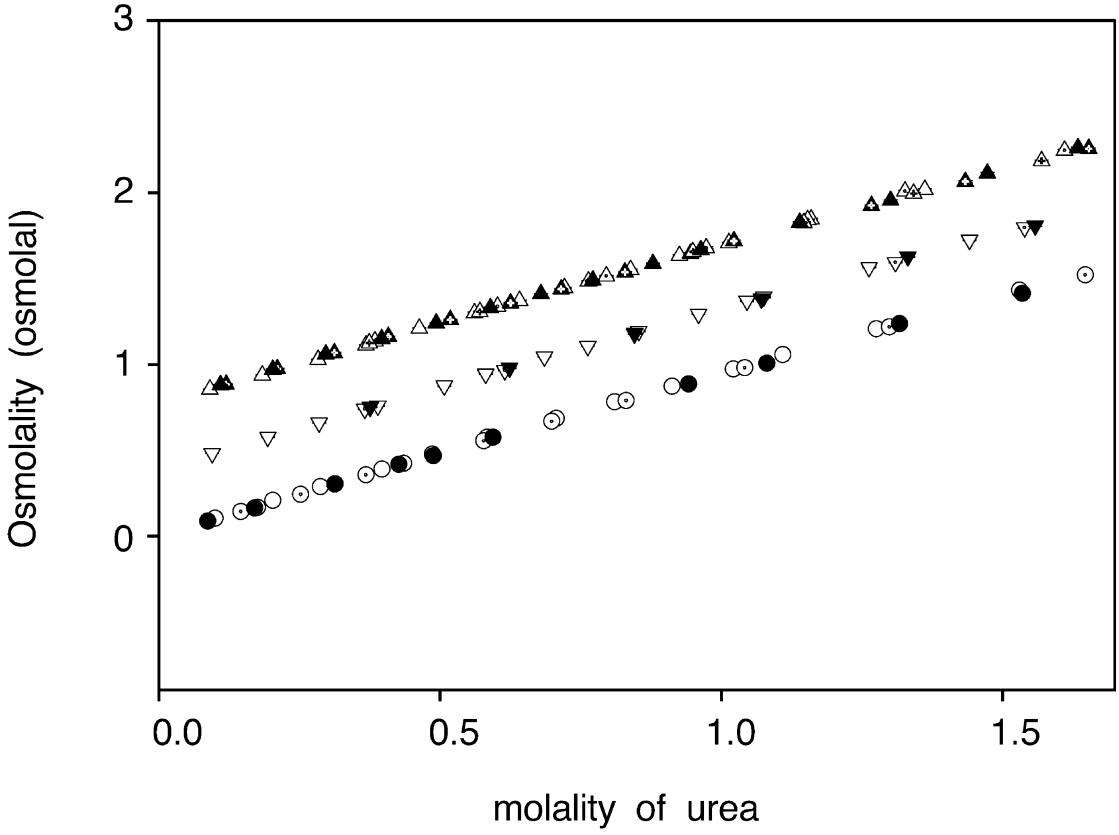


Fig. 1. VPO determinations of osmolality of H_2O–KCl–urea solutions for urea titrations at different fixed molalities of KCl (0 m, 0.212 m, 0.427 m). Osmolality is plotted vs. urea molality (m_3) for titrations at 0.427 m KCl (Δ), at 0.212 m KCl (∇) and in the absence of KCl (O). For each KCl concentration, different shadings of triangles or circles represent different data sets. Each point is the average of triplicate readings on identical samples; the uncertainty is approximately the size of the plotted point.

results but have smaller uncertainties. The error is propagated from the uncertainty in m_2 (0.002 m), reduced by a factor of $1/N^{1/2}$ because N sets of urea titration data ($N=3$ for 0.212 m KCl, $N=5$ for 0.427 m KCl) were used in global fitting.

Fig. 3 presents a plot of μ_{23}^{ex} (in units of cal mol^{-1} molal^{-1} at 25 °C) obtained directly from Eq. (6). (The observed insensitivity of Osm to the different operating temperatures of the two Wescor osmometers implies that none of the activity coefficients in this system depends significantly on temperature over the range investigated (23–37 °C). Consequently, μ_{23}^{ex} is directly proportional to absolute temperature (cf. Eq. (6)) and none of the preferential interaction coefficients has any significant temperature dependence in this range.) Values of μ_{23}^{ex} at each fixed m_2 exhibit no significant dependence on m_3 and are the same within uncertainty at 0.212 and 0.427 m KCl. Therefore, all values of μ_{23}^{ex} over the experimental concentration range of urea at both KCl concentrations were averaged (weighted by uncertainty as shown in Eq. (16)) to obtain the value $\mu_{23}^{ex}=-7.3\pm0.5$ cal mol^{-1} molal^{-1}, which is plotted as the horizontal line shown in Fig. 3. Although propagated uncertainties in individual values of μ_{23}^{ex} have magnitudes as large as μ_{23}^{ex}, the standard deviation of the mean value of μ_{23}^{ex} is much smaller (±0.5 cal mol^{-1} molal^{-1}). Fig. 3 also shows the contribution to μ_{23} from ideal mixing entropy (μ_{23}^{mix}),

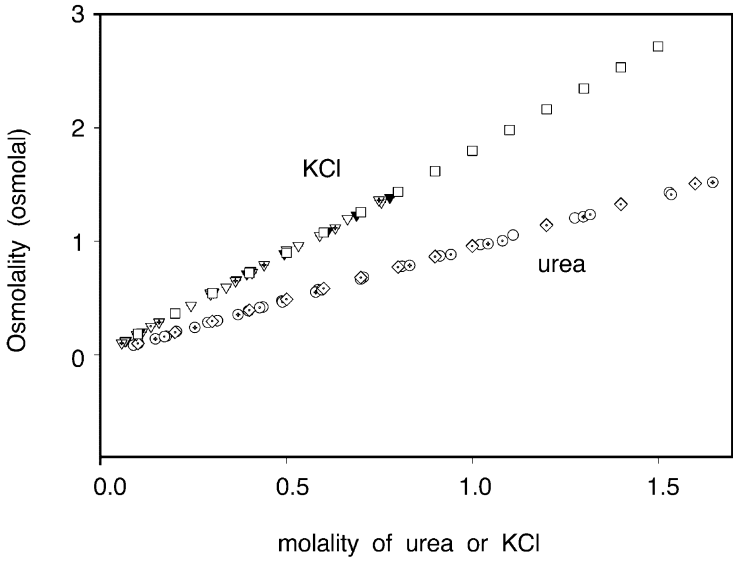


Fig. 2. Osmolalities of two component urea or KCl solutions as a function of urea or KCl molality. VPO data for two-component urea solutions (O) are compared with literature data (◇, [13], [22]). VPO data for two-component KCl solutions (∇) are compared with fitted literature values (□, [21]). Various 'shadings' of triangles and circles represent different VPO data sets. Each VPO data point is the average of triplicate readings of identical samples; the uncertainty is approximately the size of the plotted point.

calculated exactly from Eq. (7). Over the range of m_3 examined (up to 1.5 molal), μ_{23}^{mix} is relatively constant at ~ -20.7 cal mol^{-1} molal^{-1} and clearly makes the major contribution to μ_{23}. The negative sign of μ_{23}^{ex} implies favorable interactions between components 2 and 3, but the small magnitude of this derivative indicates that these weak interactions are only slightly favorable as compared with a hypothetical system in which the two solute components interact only with water.

Values of the coefficient $\Gamma\mu_3$ for urea–KCl interactions are required for our analyses of the effects of changing urea concentration on protein-DNA binding in the presence of KCl (Hong et al., in preparation). In Fig. 4 values of $\Gamma\mu_3$ calculated using Eq. (2) are plotted vs. m_3 with error bars calculated by propagation of uncertainties in m_2 and in the fitting parameters α_1, α_2, α_3 (shown in Table 1). All values of $\Gamma\mu_3$ are positive, increase with increasing m_3, and are approximately the same at 0.212 and 0.427 m KCl. As indicated by Fig. 4, $\Gamma\mu_3$ varies linearly with m_3 and approaches 0 when m_3 decreases toward 0. On the basis of Eq. (2), $\Gamma\mu_3$ must be 0 at $m_3=0$ whenever the

Table 1
Concentration dependence of osmolality in aqueous solutions of urea and KCl

Concentrations (molal)		Fitting function: $Osm=\alpha_1+\alpha_2 m_i+\alpha_3 m_i^2$ ($i=2$ or 3)					
KCl	Urea	α_1	α_2	α_3	Covariance ($\times 10^{-5}$)		
					$2\sigma^2_{\alpha_1\alpha_2}$	$2\sigma^2_{\alpha_1\alpha_3}$	$2\sigma^2_{\alpha_2\alpha_3}$
0.212	0.3–1.56	0.384 ± 0.004	0.975 ± 0.011	-0.0361 ± 0.0058	−9.0	4.7	−12
0.427	0.09–1.6	0.767 ± 0.001	0.963 ± 0.005	-0.0340 ± 0.0028	−1.2	0.66	−2.5
0	0.17–1.65	-0.005 ± 0.002	0.995 ± 0.005	-0.0423 ± 0.0030	−1.4	0.80	−2.9
0.06–0.8	0	0.004 ± 0.001	1.812 ± 0.009	-0.0316 ± 0.0118	−2.0	2.3	−20

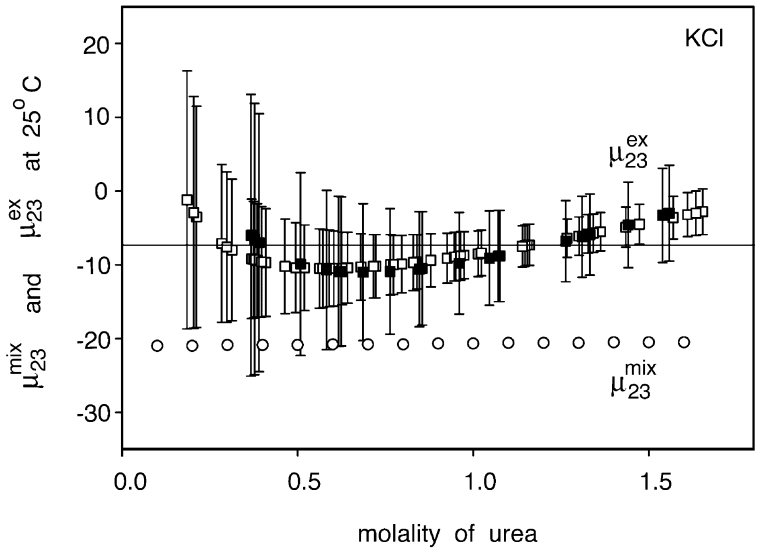
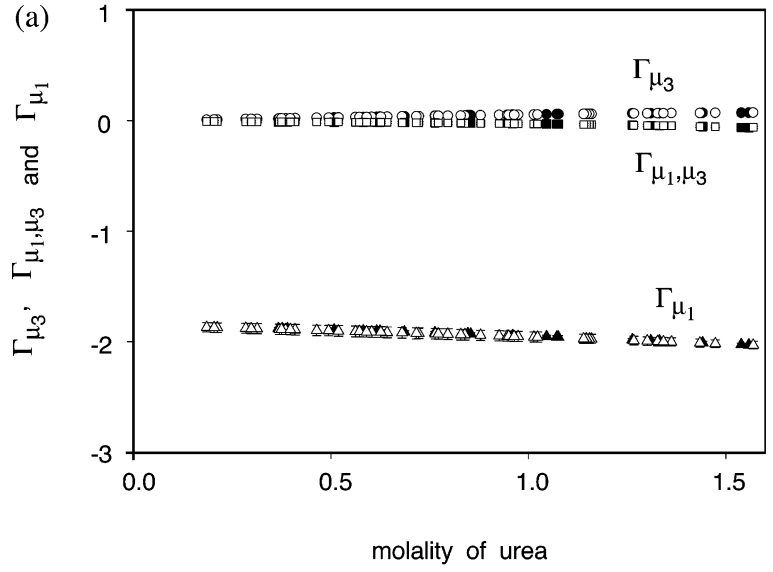


Fig. 3. Values of the ideal mixing and excess chemical potential derivatives μ_{23}^{mix} (O) and μ_{23}^{ex} (□, ■) at 25 °C for urea–KCl solutions at KCl concentrations of 0.212 (■) and 0.427 (□) molal as functions of urea molality (m_3). The horizontal line is the error weighted average of μ_{23}^{ex}, calculated using Eq. (16), at both 0.212 and 0.427 molal KCl over the concentration ranges of urea examined. Values of μ_{23}^{mix} are calculated from Eq. (7); corresponding values of μ_{23}^{ex} from Eq. (6). Error bars are propagated from the error in m_2 (reduced by a factor of $1/N^{1/2}$ because N data sets were used in global fitting) and in fitting parameters shown in Table 1. Units for μ_{23}^{mix} and μ_{23}^{ex} are cal mol^{-1} molal^{-1}.

perturbing solute (component 3) is, like urea, a nonelectrolyte [10]. Therefore, a linear function of m_3 with intercept fixed at zero was used to fit globally all values of $\Gamma\mu_3$ at 0.212 and at 0.427 m KCl. The F_R-test and the multiple-correlation coefficient R^2 [20] demonstrate the good quality of this fitting. The error weighted best fitting gives $\Gamma\mu_3 = (0.052 \pm 0.001)m_3$. With regard to Eq. (2), the linearity of $\Gamma\mu_3$ with m_3 can be rationalized by noting that μ_{23} is essentially independent of m_3 (as shown in Fig. 3) and that μ_{33} for urea is approximately inversely proportional to m_3 over a wide concentration range, consistent with the two component data ($m_2 = 0$) in Fig. 1, which show that $(\partial \mathrm{Osm}/\partial m_3)_{T,P}$ is approximately constant.

For the interactions of a biopolymer with a small solute that have been investigated so far [5,7], $\Gamma\mu_1,\mu_3$ is equal to $\Gamma\mu_3$ within experimental uncertainty, whereas for interactions of two small solutes, NaCl and urea, the difference between $\Gamma\mu_1,\mu_3$ and $\Gamma\mu_3$ is significant, and increases with increasing urea concentration, as shown by Fig. 2 in Ref. [11]. For the water–KCl–urea system we here calculate $\Gamma\mu_1,\mu_3$ so that comparisons of this coefficient with $\Gamma\mu_3$ can be made for solutions in which component 2 is either KCl, NaCl or the protein BSA. For applications of Eqs. (9a) and (9b) to calculate values of $\Gamma\mu_1,\mu_3$, corresponding values of $\Gamma\mu_1$ were calculated first using Eq. (11) as described in Section 3. This approximate way of calculating $\Gamma\mu_1$ as a function of m_3 does not introduce a significant source of error, because at each fixed osmolality investigated here, minimal curvature of m_3 vs. m_2 is apparent over the range of m_2 from 0 to 0.427 m. As indicated by Fig. 4a, at the two KCl concentrations, the estimated values of $\Gamma\mu_1$ do not differ outside of experimental uncertainties. Over the urea concentration range examined, the values of $\Gamma\mu_1$ are approximately −1.9, which differs by only 5% from the value corresponding to an ideal dilute solution ($\Gamma\mu_1 = -2$), in which $\mu_{ij}^{ex} = 0$ for all components i and j.

The values of $\Gamma\mu_1,\mu_3$ plotted vs. m_3 in Fig. 4 are calculated with Eqs. (9a) and (9b) with corresponding values of $\Gamma\mu_3$ obtained from Eq. (2) and the ΔOsm approximation (Eq. (6)). Also in



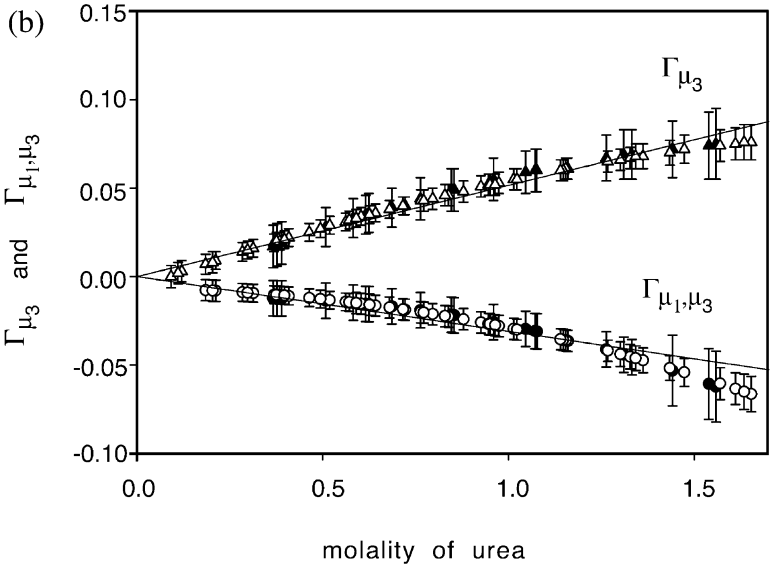


Fig. 4. (a) Comparison of $\Gamma\mu_1$ (triangles), $\Gamma\mu_3$ (circles) and $\Gamma\mu_1,\mu_3$ (squares) for urea–KCl interactions at 0.212 m (closed symbols) and 0.427 m KCl (corresponding open symbols). Values of $\Gamma\mu_1$, $\Gamma\mu_3$ and $\Gamma\mu_1,\mu_3$ calculated as described in the text are plotted against urea molality. The uncertainty, calculated as described in the text, is approximately the size of the plotted points. (b) Comparison of $\Gamma\mu_3$ (triangles) and $\Gamma\mu_1,\mu_3$ (circles) for KCl–urea interaction at 0.212 m KCl (closed symbols) and 0.427 m KCl (corresponding open symbols) over the concentration range of urea examined. The lines are error weighted global linear fittings for $\Gamma\mu_3$ or $\Gamma\mu_1,\mu_3$ at both 0.212 m and 0.427 m KCl, with intercept forced at zero: $\Gamma\mu_3/m_3 = 0.052 \pm 0.001$ and $\Gamma\mu_1,\mu_3/m_3 = -0.031 \pm 0.001$.

Eq. (9b) $\bar{V}_1$ is approximated by the molar volume of pure water (0.01807 l mol^{-1}) and $\bar{V}_3$ by the partial molar volume of urea in dilute two-component aqueous solutions: 0.044(23) l mol^{-1} [6,23,24]. The plots in Fig. 4a show that $\Gamma\mu_1,\mu_3$ is more similar to $\Gamma\mu_3$ than to $\Gamma\mu_1$. Nevertheless, the systematic difference between $\Gamma\mu_3$ and $\Gamma\mu_1,\mu_3$ falls outside the respective experimental uncertainties of these coefficients, as shown in Fig. 4b. The functional dependence of $\Gamma\mu_1,\mu_3$ on m_3 can be well described as linear with zero intercept, as shown by the quality of the fitting in Fig. 4. On the basis of Eq. (9a), $\Gamma\mu_1,\mu_3$ must have zero intercept for these solutes because $\Gamma\mu_3$ does. The error weighted best fitting gives $\Gamma\mu_1,\mu_3 = -(0.031 \pm 0.001)m_3$.

5. Discussion

5.1. Comparison of chemical potential derivatives μ_{23}^{ex} for KCl–urea, NaCl–urea and protein–urea interactions

The derivative μ_{23}^{ex} (Eq. (6)) provides a fundamental measure of thermodynamic consequences of interactions between solutes. For unfavorable interactions (relative to interactions with water) $\mu_{23}^{ex} > 0$; for favorable interactions $\mu_{23}^{ex} < 0$. If $\mu_{23}^{ex} = 0$, then $\mu_{23} = \mu_{23}^{mix}$, which describes the small effect of changing urea molality on the mole fractions of K^+ and Cl^- ions (cf. Eq. (7)). One measure of the strength of solute–solute interactions is obtained by comparing the magnitudes of μ_{23}^{ex} and μ_{23}^{mix}. If $|\mu_{23}^{ex}| \gg |\mu_{23}^{mix}|$, interactions between two solutes are strong and determine the sign and magnitude of $\mu_{23} = \mu_{32}$. This derivative in turn determines the sign of $\Gamma\mu_3$, as indicated by Eq. (2), because the sign of μ_{33} must be positive. Interactions between components 2 and 3 can be characterized as 'weak' for systems in which $|\mu_{23}^{ex}| \lesssim |\mu_{23}^{mix}|$.

In aqueous solutions of KCl and urea at 25 °C, $\mu_{23}^{ex} \cong -7$ cal mol^{-1} molal^{-1} and $\mu_{23}^{mix} \cong -21$ cal mol^{-1} molal^{-1} over the ranges of m_3 and m_2 investigated. The contribution to μ_{23} from interactions of KCl and urea is therefore small compared with the contribution from ideal mixing of these solute species. Furthermore, μ_{23}^{ex} is smaller in magnitude than either $(\mu_{22}^{ex})^{o(3)}$ or $(\mu_{33}^{ex})^{o(2)}$. For two-component aqueous solutions containing KCl or urea, analysis of the observed dependence of Osm on solute concentrations shows that $(\mu_{22}^{ex})^{o(3)}$ ranges from -550 cal mol^{-1} molal^{-1} at 0.2 molal KCl to -260 cal mol^{-1} molal^{-1} at 0.4 m KCl, and that $(\mu_{33}^{ex})^{o(2)}$ ranges from -100 cal mol^{-1} molal^{-1} at 0.2 molal urea to -40 cal mol^{-1} molal^{-1} at 1.5 molal urea. Therefore, for two-component solutions in the concentration ranges of interest here, the effect of KCl concentration on the excess chemical potential of KCl $(\mu_{22}^{ex})^{o(3)}$ far exceeds the effect of urea concentration on the excess chemical potential of urea $(\mu_{33}^{ex})^{o(2)}$, which in turn greatly exceeds the effect of the concentration of either solute on the excess chemical potential of the other $(\mu_{23}^{ex} = \mu_{32}^{ex})$ in solutions containing both.

Bower and Robinson [13] analyzed their extensive set of ID data, consisting of 66 data points for aqueous solutions of NaCl and urea over a very wide range of concentrations of NaCl (up to 6.2 molal) and urea (up to 22 molal). All but three of these ID data points pertain to concentrations of NaCl and/or urea that exceed the ranges examined here for KCl and urea. (The water–KCl–urea system has not been investigated by ID.) For the water–NaCl–urea system, the entire data set was analyzed [13] by a modification of the ΔOsm approximation [12] in order to calculate the mean ionic activity coefficient of NaCl and the activity coefficient of urea in aqueous solutions as functions of m_{NaCl} and m_{urea}. (These activity coefficients, unlike that in our Eq. (6), are defined on the molal scale.) For this system, we calculate values of μ_{23} at 25 °C by differentiating with respect to m_{urea} the polynomial function representing the mean ionic activity of NaCl in terms of powers of m_{NaCl} and m_{urea} with the expansion coefficients reported in Ref. [13]. After subtracting μ_{23}^{mix} (-20.7 cal mol^{-1} molal^{-1}, as for KCl–urea), we obtain the values of μ_{23}^{ex} in the range $0.1 < m_3 < 1.6$ m that are shown in Fig. 5: 1–4 cal mol^{-1} molal^{-1} for 0.21 m NaCl and 3–6 cal mol^{-1} molal^{-1} for 0.43 m NaCl. The reliability of these results may be affected at these concentrations (accessible to VPO), because they fall at

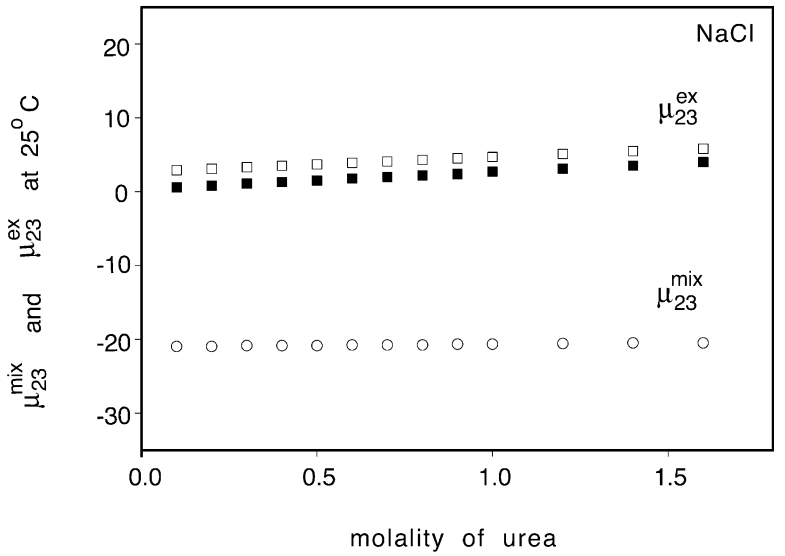


Fig. 5. Contributions of the ideal mixing entropy (μ_{23}^{mix}) and interactions (μ_{23}^{ex}) to the chemical potential derivative μ_{23} at 25 °C for H_2O–NaCl–urea solutions as functions of urea concentration at NaCl concentrations of 0.21 and 0.43 molal. Circles (O) represent μ_{23}^{mix}; squares μ_{23}^{ex}, with (□) for 0.43 m NaCl and (■) for 0.21 m NaCl, calculated as described in the text from the fitting equation in Ref. [13]. Units for μ_{23}^{mix} and μ_{23}^{ex} are cal mol^{-1} $molal^{-1}$.

one end of the much more extensive range covered by the ID data that were fitted in order to obtain the expansion coefficients [13]. Even with this potential uncertainty, the conclusion is that for NaCl–urea, as for KCl–urea, μ_{23}^{ex} makes a substantially smaller contribution to μ_{23} than does μ_{23}^{mix}.

For either KCl or NaCl in aqueous urea solutions, our best estimate of $|\mu_{23}^{ex}|$ is less than half as large as $|\mu_{23}^{mix}|$; in both cases μ_{23}^{ex} is close to zero over the ranges of m_3 and m_2 examined. Although uncertainties in μ_{23}^{ex} are large, our experimental data indicate that KCl–urea interactions in water are marginally favorable, whereas our analysis of the ID measurements [13] indicates that NaCl–urea interactions in water are marginally unfavorable. This relative ordering of μ_{23}^{ex} is consistent with the more negative hydration free energy of Na^+ compared with K^+ [25–27]. Moreover, the finding that both NaCl and KCl exhibit $\mu_{23}^{ex} \cong 0$ for interactions with urea is consistent with the relatively weak interactions found for NaCl and KCl (compared to other salts) with acrylamide functional groups on a polyacrylamide column [28], and with the placement of both NaCl and KCl together in mid-range (relatively nonperturbing) positions of the Hofmeister series of salts ranked in order of their effects on protein (polyamide) processes that change the exposure of protein surface to aqueous solution [29–31].

For interactions in aqueous solution containing urea and native BSA, a large protein that has $\sim 2.9 \times 10^4$ Å^2 of water accessible surface area (ASA), published values of $\Gamma\mu_3$ [5] yield $\mu_{23}^{ex} = -3.3 \pm 1.1$ kcal mol^{-1} $molal^{-1}$ at 25 °C for $m_3 \leqslant 1$ molal. Thus, BSA interactions with urea have large favorable thermodynamic effects (compared with a hypothetical solution in which each solute interacts only with water). Ideal mixing effects are size-independent, and applications of Eq. (7) show that $\mu_{23}^{mix} \sim -21$ cal mol^{-1} $molal^{-1}$ for BSA–urea, KCl–urea and NaCl–urea in aqueous solutions. Because μ_{23}^{ex} greatly exceeds μ_{23}^{mix} for BSA–urea interactions, the sign and magnitude of μ_{23} are determined by μ_{23}^{ex}, which is at least ~ 450 times larger for BSA–urea than for KCl–urea.

For the interactions of a solute with members of a homologous series of proteins, $\Gamma\mu_1,\mu_3$ is

predicted to be proportional to the water ASA of the protein [5,6]. Because $\Gamma\mu_{1},\mu_{3}$ is related directly to $\Gamma\mu_{3}$ (via Eq. (9a)) and hence to μ_{23}^{ex} (via Eq. (2)), values of μ_{23}^{ex} normalized by ASA may afford a more informative basis for comparing thermodynamic effects due to biopolymer–urea interactions with those due to salt–urea interactions. The relevant (average) ASA of the two salt ions depends on whether urea can replace first shell hydration water. Using accepted values [32] for the unhydrated ionic radii of K^{+}, Cl^{-} and Na^{+} (1.33, 1.81, 0.95 Å, respectively), we obtain values of μ_{23}^{ex}/ASA of 0.06, −0.11 and −0.11 cal mol^{-1} $molal^{-1}$ $Å^{-2}$ for interactions of NaCl, KCl and BSA, respectively, with urea. Thus, values of μ_{23}^{ex}/ASA for interactions of KCl and of BSA with urea in solution appear to be similarly favorable whereas the NaCl–urea interaction appears slightly unfavorable. Use of larger (hydrated) radii for K^{+} and Cl^{-} ions might introduce a significant difference between values of μ_{23}^{ex}/ASA for BSA–urea interactions and KCl–urea interactions.

5.2. Differences between $\Gamma\mu_{3}$ and $\Gamma\mu_{1},\mu_{3}$ for systems in which interactions are weak: non-negligible contributions of ideal mixing and osmotic terms

For the interactions of KCl (or NaCl) and urea in water, many characteristics of $\Gamma\mu_{3}$, $\Gamma\mu_{1},\mu_{3}$ and $\Gamma\mu_{1}$ are consistent with those found for the interactions in solutions where component 2 is BSA rather than a simple salt, and component 3 may be any of a wide variety of biologically relevant solutes [5,7]. Thus, for interactions in solutions containing either a 1:1 salt or BSA and urea at relatively low m_{2} and m_{3}, both $\Gamma\mu_{3}$ and $\Gamma\mu_{1},\mu_{3}$ are independent of m_{2} and proportional to m_{3}, whereas $\Gamma\mu_{1}$ is independent of m_{2} and a linear function of m_{3} with a negative intercept.

Apparent differences in the characteristics of preferential interaction coefficients for salt–urea interactions as compared with biopolymer–urea interactions are that $(\Gamma\mu_{3}/m_{3})>0>(\Gamma\mu_{1},\mu_{3}/m_{3})$ for interactions of KCl or NaCl and urea, whereas $(\Gamma\mu_{3}/m_{3})\cong(\Gamma\mu_{1},\mu_{3}/m_{3})\gg 0$ for interactions of BSA and urea. These differences should be considered in the context of large differences in the experimental uncertainties as compared with the magnitudes of these coefficients. For BSA–urea, values of $\Gamma\mu_{3}$ and $\Gamma\mu_{1},\mu_{3}$ are relatively large, but the experimental uncertainties also are large in an absolute sense: $(\Gamma\mu_{3}/m_{3})\cong(\Gamma\mu_{1},\mu_{3}/m_{3})=6\pm 2$. The KCl–urea interaction is weak in the sense that $|\mu_{23}^{ex}|<|\mu_{23}^{mix}|$, but both $\Gamma\mu_{3}$ and $\Gamma\mu_{1},\mu_{3}$ are determined with sufficient accuracy to show that these coefficients have opposite signs ($\Gamma\mu_{3}/m_{3}=0.052\pm 0.001$; $\Gamma\mu_{1},\mu_{3}/m_{3}=-0.031\pm 0.001$). Hence, for KCl–urea solutions the difference $(\Gamma\mu_{3}-\Gamma\mu_{1},\mu_{3})/m_{3}\cong 0.083$ is smaller by more than an order of magnitude than the uncertainty in $\Gamma\mu_{3}/m_{3}$ or $\Gamma\mu_{1},\mu_{3}/m_{3}$ for BSA–urea solutions, where the difference $\Gamma\mu_{3}-\Gamma\mu_{1},\mu_{3}$ is several times larger than that for KCl–urea solutions, but does not lie outside the overlapping uncertainties in $(\Gamma\mu_{3}/m_{3})$ and in $(\Gamma\mu_{1},\mu_{3}/m_{3})$ for the BSA–urea interaction.

Because μ_{23}^{mix} is at least twice as large in magnitude as μ_{23}^{ex} for KCl– (or NaCl–) urea interactions, neither $\Gamma\mu_{3}$ nor $\Gamma\mu_{1},\mu_{3}$ is as straightforward as μ_{23}^{ex} to interpret at a thermodynamic or molecular level. In particular, the negative values of $\Gamma\mu_{1},\mu_{3}/m_{3}$ for both salts do not imply unfavorable interactions with urea, but rather must be understood as resulting from the net consequences of interactions, ideal mixing effects, and osmotic pressure effects. In contrast, for BSA–urea interactions $\mu_{23}\cong\mu_{23}^{ex}$, and the numerically similar coefficients $\Gamma\mu_{3}$ and $\Gamma\mu_{1},\mu_{3}$ are directly interpretable measures of the thermodynamic and molecular consequences of the BSA–urea interaction.

As shown in Hong et al. (in preparation), preferential interactions of salt and urea in water–salt–urea systems must be considered to analyze experimental values of the derivative $\partial\ln K_{obs}/\partial m_{3}$ that expresses the effect of urea concentration on K_{obs} for a biopolymer process such as protein-DNA binding in a KCl solution. In the expression resulting from this analysis, the value of $\Gamma\mu_{3}/m_{3}$ for KCl–urea interactions is weighted by the net charge on the DNA binding site of a protein ligand. Because results reported here show that $\Gamma\mu_{3}/m_{3}$ for KCl–urea solutions is small in magnitude $(\Gamma\mu_{3}/m_{3}=0.052\pm 0.001)$, the contribution of salt–urea interactions to the derivative $\partial\ln K_{obs}/\partial m_{3}$ is invariably negligible in comparison to

typical experimental uncertainties. The same conclusion applies for solutions containing NaCl instead of KCl.

Our previous analysis of an extensive range of ID data for H_2O–NaCl–urea indicates that $\Gamma\mu_3 \cong 0$ (Fig. 2 in Ref. [11]). For NaCl–urea solutions in the range of interest here, we used Eq. (2) to calculate $\Gamma\mu_3$ from μ_{23} (as in Fig. 5) and μ_{13} (which is calculated by fitting Osm as a function of m_2 and m_3 as in Ref. [11]). We obtain $\Gamma\mu_3/m_3 \cong 0.03$ for solutions in which $m_2 = 0.21$ m or 0.43 m NaCl over the range $0.1 < m_{\text{urea}} < 1.6$ m. This result is consistent, within uncertainty, with the calculations presented in Fig. 2 of Ref. [11] over a much wider range of m_3, with m_2 fixed at a representative mid-range value (2 molal). In summary, the calculations reported in the present work indicate that, when either KCl or NaCl is used as the electrolyte in investigations of urea effects on biopolymer processes, the dependence of K_{obs} on urea molality can be analyzed without significant correction for effects due to salt–urea interactions.

Acknowledgments

We thank Daniel J. Felitsky and Jonathan Cannon for helpful discussions and useful data for comparison, and Sheila Aiello for preparation of the manuscript. This research was supported by NIH Grant GM 47022.

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