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ACTIVITY COEFFICIENTS OF SALTS IN HIGHLY CONCENTRATED PROTEIN SOLUTIONS. I. ALKALI CHLORIDES IN ISOIONIC BOVINE SERUM ALBUMIN SOLUTIONS

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In order to understand the thermodynamic state of simple salts in living cells, the mean activity coefficients of LiCl, NaCl, KCl, RbCl, CsCl were determined in concentrated isoionic bovine serum albumin (BSA) solutions by use of the EMF method with ion exchange membrane electrodes. The protein concentration range extended up to 22 wt %, whereas the salt concentration was kept constant at 0.1 mole per kilogram water. These solutions may be regarded as crude but appropriate model systems for the cytoplasm of cells as far as type and magnitude of the macromolecular component influence on the chemical potential of the salts is concerned. The mean stoichiometric activity coefficients of the alkali chlorides in the isoionic BSA solutions decreased linearly with the protein molality; this decrease, however, did not exceed ca. 10% compared with the pure 0.1 molal salt solutions. Only very small differences in the behaviour of the different alkali chlorides were observed. The results may be interpreted by the superposition of the effects of specific Cl^- ion binding to BSA and BSA bound "non-solvent" water with probably electrostatic long range interactions of the $BSA(Cl^-)_\nu$ polyions with the salt ions in solution. The resulting mean activity coefficients, corrected for ion binding and non-solvent water, showed a very slight linear dependence on the protein concentration. The departure from the value in the pure 0.1 molal salt solutions did not exceed ± 2%.

1. Introduction

The driving force for the diffusion and for the transport of molecules and ions in the cytoplasm and across the cell membrane of a living cell is determined by the gradient of the chemical potential of these molecules and ions and the other coupled components in the cytoplasm or across the cell membrane. The chemical potential or the activity of a component in the cytoplasm – in the large hyaloplasmic compartment or in the much smaller cell organelles, compartmentalized by intracellular membranes from the generally large hyaloplasmic compartment – depends both on the concentration of the component and on the type and magnitude of the interactions between the molecules or ions of that component with the molecules or ions of all other components in this compartment. Conventionally, the magnitude of the interaction is taken into account by the activity coefficient.

From the chemical composition of the cytoplasm [1] one may conclude that water, cell proteins and simple salts are the main components determining essentially the physico-chemical state of the cytoplasm, especially of the large hyaloplasmic compartment. Despite their small weight percentage in the cell, the simple salts and their ion constituents – like Na^+, K^+, Cl^-, phosphates etc. – are of major importance for the colligative properties of the hyaloplasm because of their large contribution to the molar composition.

The simple aqueous protein-salt-solution used and described in this paper resembles in a rather crude way the situation in a hyaloplasm as far as the high protein concentration is concerned. The first part of these studies presented here, deals with the investigation of the lyotropic alkali ion series in isoionic bovine

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serum albumin (BSA) solutions, using LiCl, NaCl, KCl, RbCl, and CsCl as salts. The salt concentration was kept constant at 0.1 mole per kilogram water. The protein concentration extended up to 22 wt %. The chemical potential of the salt in the protein-salt-solution is expressed in terms of its concentration and activity coefficient.

2. Experimental section

2.1. Materials and solutions

Reference solutions. The pure alkali chloride solutions used as reference solutions were prepared from reagent grade dried salts (MERCK, p.A.) and bidestilled deionized water. The molalities (0.07 to 0.15 moles per kilogram water) were adjusted to an accuracy of $< \pm 0.1\%$.

Isoionic BSA-salt solutions. The BSA stock solutions (8–10 wt%) were prepared from commercially available preparations ("Serumalbumin vom Rind, trocken, reinst", Behringwerke AG., Marburg, FRG) with bidestilled deionized water. They were passed twice through a mixed bed ion exchange column (SERDOLIT M-B) in order to remove foreign salts. BSA solutions of higher concentrations up to 22 wt % were produced by ultrafiltration technique (Sartorius Membranfilter GmbH., Göttingen, FRG).

The molal protein concentration of the final solution was determined by the dry weight method (24 hr at 110°C) and in using a value of 66.210 kilogram per mole for the molar mass M_p of BSA [2], to an accuracy of $< \pm 0.1\%$. Finally, the alkali chloride molality was adjusted to 0.1 moles per kilogram water by adding the appropriate quantity of the respective dried salt. The accuracy of the salt molality was $\pm 0.1\%$.

As could be verified by gel chromatography on SEPHADEX G-150, the BSA salt solutions consisted of 87% monomeric fraction and of about 10% dimeric fraction and approximately 3% higher BSA polymers. This composition is found in most BSA preparations [3,4]. Impurities possibly present like α_2-globulin, transferrin or haptoglobin [3] have been disregarded in the subsequent discussion. The dimeric portion was not taken into account, thus considering the whole BSA as a monomeric fraction.

2.2. Determination of activity coefficients

The mean activity coefficient of the alkali chlorides in the BSA salt solutions were determined by means of electromotive force (EMF) measurements in a three-compartment electrochemical cell with ion exchange membranes, applying the "zero titration" method of Sollner [5].

Instruments. Fig. 1 shows the longitudinal section of the electrochemical cell used. The ion exchange membranes had been charged with the respective ion species and equilibrated in 0.1 molal MCl solution before measurement. All compartments were equipped with electromechanical stirring in order to achieve rapid equilibrium at the membrane interfaces and reduction of the film potentials.

The reference solutions were connected to the input resistance ($10^{11}\ \Omega$) of a vibrating reed electrometer (FHT 408 A, Frieseke & Hoepfner GmbH., Erlangen, FRG) through salt bridges (KCl saturated in agar-agar) and Thalamid electrodes (Schott & Gen., Mainz, FRG). The EMF values were recorded to an accuracy of ± 0.15 mV with a potentiometric recorder (SERVOGOR, Metrawatt GmbH., Nürnberg, FRG).

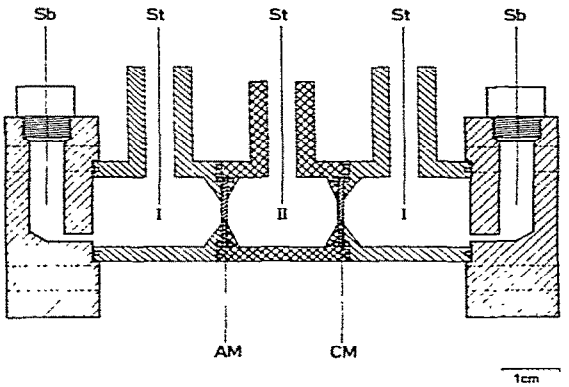

Fig. 1. Longitudinal section through the three-compartment cell used for electrochemical titration. I: compartments for the pure alkali chloride reference solutions of identical concentration; II: compartment for the BSA alkali chloride solution; AM, CM: anion and cation exchange membranes (NEPTON AR-111 and Cr-61, Ionics, Watertown, Mass., U.S.A.) respectively; St: holes for filling and stirring; Sb: holes for inserting the salt bridges; material: lucite, with silicon rubber gaskets.

Electrochemical cell and preamplifier had been installed in a Faraday cage. All measurements were done at room temperature (23 ± 1°C).

Procedure. The EMF of the electrochemical cell is given as a function of the mean ionic activities of the salt in the compartments I and II by the relation [5, 6,7]:

$$E = 2(RT/F)(1 - t_+ - t_-)\ln(a/a'), \quad (1)$$

where E means the EMF, F the Faraday constant, R the universal gas constant, T the absolute temperature and a' and a the mean ionic activity of the neutral salt component MCl (M = Li, Na, K, Rb, or Cs) in the compartments I and II respectively. t_+ and t_- represent the mean respective coion transference numbers in the ion exchange membranes and take into account the non-ideality of the membranes.

For $E = 0$, one obtains from eq. (1):

$$a = a'_0, \quad (2)$$

where a'_0 is the value for a' at $E = 0$. By use of the relation

$$a = \gamma m, \quad (3)$$

the stoichiometric mean ionic activity coefficient γ of MCl in the protein solution is obtained:

$$\gamma = a'_0/m. \quad (4)$$

(For simplicity, the subscript ± of the conventionally used symbol $\gamma_\pm$ is omitted in this paper, since no confusion with symbols for other activity coefficients is possible.) As long as the variation of the salt concentration in the reference solutions is small enough as not to change significantly the coion transference numbers in the membranes, the EMF exhibits a linear dependence on $\ln a'$ according to eq. (1). (For derivation and discussion of the validity of eq. (1): see [7].)

In practice, at each composition of the protein-salt-solution a linear relationship between E and $\ln a'$ could be observed within the experimental limits of error. Fig. 2 shows a representative example.

The a' values have been calculated from the molalities and interpolated values for the activity coefficients in pure salt solutions given in the literature [8, 9]. By linear regression, a'_0 is obtained, from which, by use of eq. (4), γ could be calculated. The overall accuracy of the method is ± 0.2%.

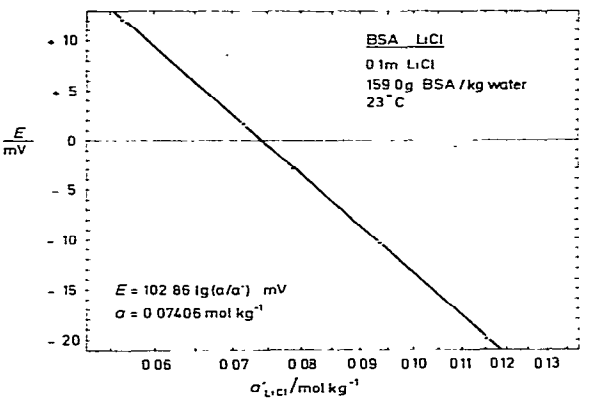

Fig. 2. Electrochemical titration of a solution in compartment II of the cell (fig. 1) containing 0.1008 mole LiCl and 159.0 grams BSA per kilogram water. Electromotive force E in millivolts versus the activity a' of LiCl in mole per kilogram water in the compartments I of the cell in fig. 1. From $a' = 0.07406$ mol kg^{-1} at $E = 0$ and $m = 0.1008$ mol kg^{-1}: $\gamma = 0.7347$, according to eq. (4).

3. Results

In fig. 3, the mean stoichiometric activity coefficients of the alkali chlorides LiCl, NaCl, KCl, RbCl, and CsCl in isoionic BSA solutions are shown in dependence on the BSA molality. The total salt concentration was kept constant at 0.1 moles per kilogram water. Data points for KCl from a previous investigation [7,10] are included.

The stoichiometric mean activity coefficients, calculated from a'_0 by means of the total ion concentrations according to eq. (4), reflect the influence of all mutual interactions between the components of the solution and the salt. To arrive at a more direct measure of the interactions which are caused by the addition of increasing amounts of isoionic BSA to a 0.1 molal salt solution, the normalized ratio γ/γ^0 is plotted versus BSA molality in fig. 4, where γ^0 represents the mean activity coefficient of the respective salt in pure 0.1 molal salt solution [8,9].

Above all, these results show that the presence of BSA causes a linear but relatively slight decrease of γ in relation to γ^0 for all alkali chlorides. Even at a BSA concentration of 4.0 millimoles per kilogram water, which equals 20.94 wt%, this decrease is only about 10%.

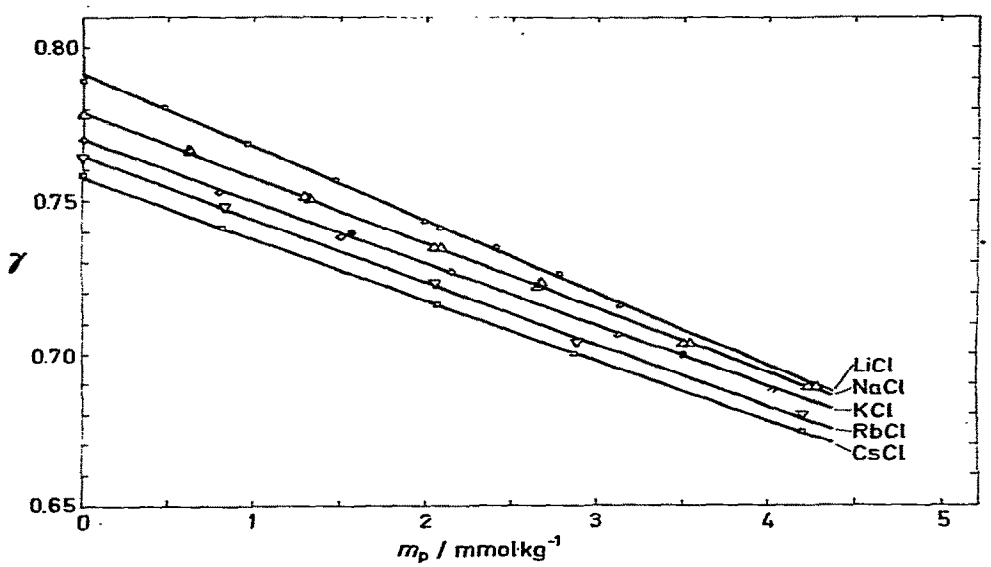

Fig. 3. Stoichiometric mean activity coefficients of LiCl (○), NaCl (△), KCl (◇), RbCl (▽) and CsCl (□) in isoionic BSA solutions in dependence on the BSA molality m_p. Salt concentrations: 0.1 mole per kilogram water. Only the mean values of γ are shown. The data points on the ordinate show the mean activity coefficients γ^0 of the pure alkali chloride solutions taken from the literature [8,9]. (◆: results for KCl from [7]).

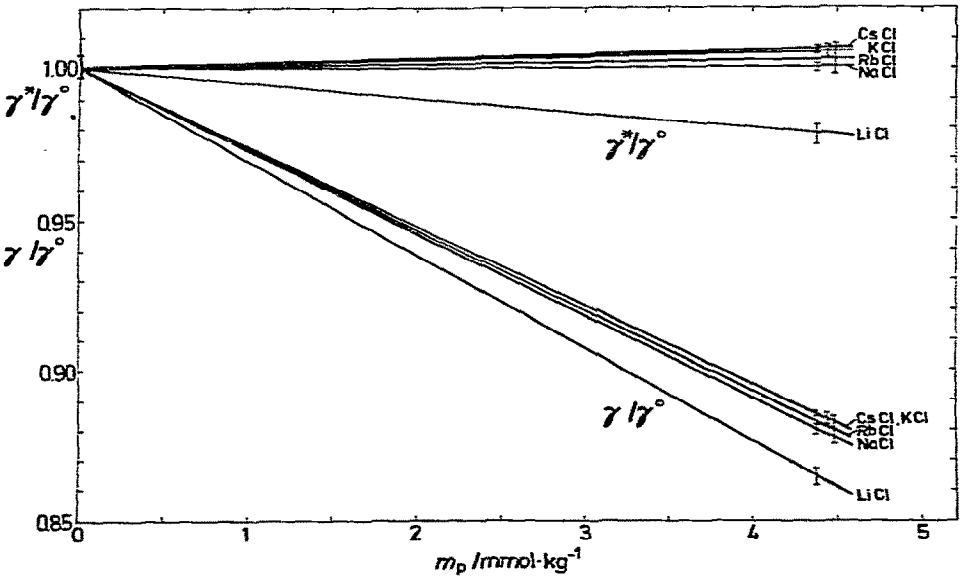

Fig. 4. Relative stoichiometric mean activity coefficients γ/γ^0 and relative mean activity coefficients corrected for "non solvent" water and Cl^- binding, γ^*/γ^0, in isoionic BSA alkali chloride solutions in dependence on the BSA molality m_p. Salt concentrations: 0.1 mole per kilogram water. "Non solvent" water: 0.3 gram per gram dry BSA; average number of bound Cl^- ions per BSA molecule: 8.1 to 9.0, depending on BSA concentration. For sake of simplicity and in order to give a clear representation of the influence of BSA on the activity coefficients of the different salts, which is given by the slopes, only the regression lines are shown in the figure. They have been parallely shifted to an exact ordinate intercept at 1, the theoretical value at zero protein concentration. The maximum deviation range of the "true" ordinate intercepts of the regression lines is shown by a bar on the ordinate in the figure (range of β_0 and β_0^* in tables 1 and 2). The bars at the regression lines correspond to the standard deviations of the data points from the regression lines (s^* in tables 1 and 2).

4. Discussion

4.1. Activity coefficients

The influence of BSA on the chemical potential of the salts is described by the change in their chemical potential when adding BSA to the pure salt solution. The chemical potential μ of the (1,1)-salt MCl (M = Li, Na, K, Rb, Cs) in a pure salt solution of molality m is

$$\mu = \mu_0 + 2RT \ln \gamma^0 m, \quad (5)$$

where μ_0 is the chemical standard potential and γ^0 the stoichiometric mean ionic activity coefficient of the salt in the pure salt solution.

After the addition of the dry salt free BSA, the chemical potential of the salt changes to μ_p,

$$\mu_p = \mu_0 + 2RT \ln \gamma m, \quad (6)$$

where γ is now the stoichiometric mean activity coefficient of the salt in the protein-salt-solution. Therefore, the change in the chemical potential of the salt upon the addition of the protein at constant salt molality m and constant temperature and pressure, $\Delta\mu = (\mu_p - \mu)$, is

$$\Delta\mu = 2RT \ln (\gamma/\gamma^0). \quad (7)$$

Thus, γ/γ^0 as given in fig. 4 for BSA alkali chloride solutions, describes the net effect of BSA on the salt.

4.2. Chloride binding and non-solvent water

The interpretation of the stoichiometric activity coefficients in terms of interactions between the solution components requires assumptions, considering the so-called "protein bound" water and the salt ions bound to BSA.

In aqueous isoionic solutions the monomeric BSA molecule may be regarded as a compact, very stable hydrated molecule [11], carrying practically no net electrical charge in the absence of salts, whereby about 100 positive and negative elementary charges being distributed nearly symmetrically over the surface of the molecule [11,12].

It is known that the BSA molecule exhibits a rather strong affinity to small anoins even at isoionic pH, whereas cations are not bound at this pH [11,13–15]. For example, about 9 Cl^- ions are bound to specific binding sites of the BSA molecule at a Cl^- molality of 0.1 in isoionic BSA solutions [15] ‡.

Furthermore, the BSA molecule, like all proteins, "binds" appreciable amounts of water on its surface [19]. In the following, it is assumed that the bound water does not contain small hydrated ions and, thus, would act as "non-solvent" water to the salt ions in solution [20,21].

Therefore, serving as a simple model for the interpretation of our results, the BSA molecule is regarded as a polyanion $BSA(Cl^-)_\nu$ with a net charge number of about −9, resulting from 9 specifically bound Cl^- ions, carrying a "hydration shell" corresponding to 0.2 to 0.5 grams of "non-solvent" water per gram dry BSA [19,22].

Thus, this simple model of the BSA salt solution assumes a concentration change in the separate kinetic units of the solution components due to the inaccessibility of the "non-solvent" water to the salt ions as well as due to the binding of chloride ions to BSA.

With m_+^* and m_-^*, the molality of "free" cations and anions of the salt in water accessible to the salt ions, eq. (7) takes the form

$$\Delta\mu = RT \ln \left(\frac{m_+^* m_-^*}{m^2} \right) + 2RT \ln \frac{\gamma^*}{\gamma^0}, \quad (8)$$

where γ^* is now the "true" mean activity coefficient of the salt.

If η means the mass of "non-solvent" water in kilograms water per mole BSA, ν the average number of

‡ The interpretation of the results gained from EMF and ΔpH measurements in dilute protein salt solutions by the specific binding of ions to the macromolecules was critically reviewed by Nagasawa et al. [16,17]. The authors pointed out that the effects observed by Scatchard and others [13–15] may be partly or totally caused by failure of the smeared charge or Debye–Hückel approximations and need not to be a result of ion binding. It seems to us, however, that the extent of the lowering of the apparent anion activity in isoionic BSA solutions, with zero net electrical charge of the protein molecules, comparable to that in other polyelectrolyte solutions with high polyion charge densities and in clear contrast to the practically unchanged apparent activity of univalent cations [7,18], cannot be interpreted without assuming specific anion binding to the BSA molecules. Therefore and for lack of unambiguous analysis, we used the Cl^- binding values computed by Scatchard without ignoring that these values may carry some degree of uncertainty.

bound chloride ions per BSA molecule, m, as mentioned before, the total salt molality and m_p the total BSA molality in the protein solution, one obtains:

$$m_+^*/m = (1 - \eta m_p)^{-1} \equiv k_w^{-1}, \quad (9)$$

$$m_-^*/m = [1 - \nu(m_p/m)]k_w^{-1} \equiv k_{Cl}^{-2}k_w^{-1}, \quad (10)$$

where k_w and k_{Cl} are functions taking into account, by definition, the binding of "non-solvent" water and that of chloride ions to BSA molecules.

Inserting eqs. (9) and (10) into eq. (8) gives by comparison with eq. (7):

$$\gamma^* = k_w k_{Cl}\gamma. \quad (11)$$

In this paper, a constant value of $\eta = 19.86$ kilogram non-solvent water per mole BSA has been chosen, irrespective of BSA molality and type of salt. This value corresponds to 0.3 gram non-solvent water per gram dry BSA. The ν-values were calculated as a function of the BSA molality [23] using data of Scatchard [15]. It was assumed that the values of ν do not depend on the type of alkali ion. In the present experiments ν varied from 9.0 at $m_p = 0.47$ mmol kg^{-1} to 8.1 at $m_p = 4.31$ mmol kg^{-1}.

According to eq. (8), the ratio γ^*/γ^0 measures the change of the chemical potential of the "free" salt ions in the "free" solvent due to the presence of BSA.

Empirically, the dependence of γ/γ^0 and γ^*/γ^0 on the BSA concentration may be described by the relations

$$\gamma/\gamma^0 = \sum_{i=0}^{\infty} \beta_i m_p^i, \quad (12)$$

$$\gamma^*/\gamma^0 = \sum_{i=0}^{\infty} \beta_i^* m_p^i. \quad (13)$$

As to our results, it could be verified that γ/γ^0 and γ^*/γ^0 are given in dependence on the protein molality by the linear portions of eqs. (12) and (13) for all salts investigated within the experimental limits of error. Tables 1 and 2 show $\beta_0, \beta_1, \beta_0^*$ and β_1^* together with their standard errors and the standard deviations of the data points from the regression lines calculated by using the experimental error values σ_γ of individual data points as statistical weights.

The slopes β_1 and β_1^* describe the influence of the protein on the activity coefficient of the salt most directly:

$$\beta_1 = \frac{1}{\gamma^0}\left(\frac{\partial\gamma}{\partial m_p}\right)_{T,p,m}, \quad (14)$$

$$\beta_1^* = \frac{1}{\gamma^0}\left(\frac{\partial\gamma^*}{\partial m_p}\right)_{T,p,m}. \quad (15)$$

Therefore, in fig. 4, only the regression lines parallelly shifted through the ordinate intercept 1 are shown. The bar at the ordinate indicates the maximum range of the "true" ordinate intercepts.

Fig. 4 shows that the mean activity coefficients γ^* of all alkali chlorides investigated are changed but to a very little extent by the presence of BSA in the 0.1 molal salt solution. This change does not exceed ± 2% even at a protein concentration of 4 millimoles per kilogram water which equals 20.94% by weight.

Despite small quantitative differences which will be discussed below, these findings are in accordance with other experimental and theoretical results [7,10, 24,25] indicating that the mean activity coefficient of a simple salt in concentrated BSA solutions pre-

Table 1
Stoichiometric mean activity coefficients of alkali chlorides in isoionic BSA solutions

Salt	n	$\overline{\sigma_\gamma/\gamma}$ (%)	β_0	σ_{β_0}	β_1 (mol^{-1} kg)	σ_{β_1} (mol^{-1} kg)	s
LiCl	23	0.15	1.0047	± 0.0006	−30.923	± 0.281	0.0028
NaCl	23	0.15	1.0013	± 0.0005	−27.168	± 0.183	0.0015
KCl	14	0.14	0.9999	± 0.0008	−26.121	± 0.295	0.0018
RbCl	8	0.18	1.0005	± 0.0015	−26.709	± 0.540	0.0045
CsCl	7	0.17	0.9989	± 0.0011	−26.089	± 0.469	0.0008

Parameters and standard errors of the regression lines for γ/γ^0 in dependence on BSA molality m_p (eq. (12)): $\gamma/\gamma^0 = \beta_1 + \beta_0 m_p$; n: number of data points, $\overline{\sigma_\gamma/\gamma}$: mean value of the relative experimental errors of single determinations of γ in %, s: standard deviation of data points from the regression lines calculated with the experimental error values of individual data points as statistical weights.

Table 2
Mean activity coefficients of alkali chlorides in isoionic BSA solutions, corrected for "non-solvent" water and ion binding

Salt	n	$\overline{\sigma\gamma^*/\gamma^*}$ (%)	β_0^*	$\sigma_{\beta_0^*}$	β_1^* (mol^{-1} kg)	$\sigma_{\beta_1^*}$ (mol^{-1} kg)	s^*
LiCl	23	0.15	1.0039	± 0.0006	−4.842	± 0.294	0.0030
NaCl	23	0.15	1.0002	± 0.0005	0.196	± 0.198	0.0016
KCl	14	0.14	0.9985	± 0.0008	1.316	± 0.316	0.0021
RbCl	8	0.18	0.9984	± 0.0016	0.748	± 0.586	0.0050
CsCl	7	0.17	0.9974	± 0.0011	1.408	± 0.509	0.0011

Parameters and standard errors of the regression lines for γ^*/γ^0 in dependence on BSA molality m_p (eq. (13)): $\gamma^*/\gamma^0 = \beta_0^* + \beta_1^* m_p$. n: number of data points, $\overline{\sigma\gamma^*/\gamma^*}$: mean value of the relative experimental errors of single determination of γ^* in %, s: standard deviation of data points from the regression lines calculated with the experimental error values of individual data points as statistical weights.

valently depends on the equivalent concentration of the BSA polyions and is changed by only about 10% even at much higher protein equivalent concentrations after allowing for "non-solvent" water and specific anion binding.

Comparison of γ^*/γ^0 to γ/γ^0 in fig. 4 reveals that most of the effect of BSA on the stoichiometric activity coefficients of the alkali chlorides is due to the strong specific anion binding to BSA, although this effect is partly concealed by the effect of "non-solvent" water which acts in the opposite direction. Similarly, in the case of concentrated isoionic hemoglobin salt solutions, it was found [26–30] that most of the change in the stoichiometric activity coefficients of simple salts, besides due to the effect of bound water, could be attributed to specific anion binding to the hemoglobin molecules, the remaining effect being of the same order of magnitude as in BSA salt solutions of correspondent charge concentration. The same interpretation possibly should apply to the activity coefficients of KCl in cytoplasm fractions [31].

By and large, in all concentrated protein salt systems investigated thus far, the whole effect of the protein on the chemical potential of the salt is rather small at salt concentrations corresponding to physiological conditions.

The effect of BSA on the activity coefficient of the salts, after allowing for "non-solvent" water and for specific ion binding, is most probably due to electrostatic interactions between the $BSA(Cl^-)_\nu$ polyions and the free alkali and chloride ions. It has been shown [7], that the application of the "cell model" theory of Marcus [32], Katchalsky and Alexandrowicz [33] and that of the Oosawa theory [34,35] to BSA-KCl solutions could describe fairly well the general trend of the experimental activity coefficients of the salt. A quantitative comparison, however, between our results and theoretical values of the activity coefficients based on the application of the electrostatic theory of polyelectrolyte solutions should be made very cautiously in view of the assumptions made in taking into account "non-solvent" water and specifc chloride binding.

Fig. 5 illustrates the influence of the correction terms k_{Cl} and k_w for ion binding and for "non-solvent" water on the final relative activity coefficient values by plotting γ^*/γ^0 for NaCl at $m_p = 4.3073$ millimole per kilogram water in dependence on ζ (ζ: mass of "bound" water per mass of dry BSA; $\zeta = 1000\ \eta/M_p$) with various ν-values as parameters. As can be seen, the assumed uncertainties of ν and ζ values cause a maximum uncertainty of γ^*/γ^0 of +6.5% and −6.1% considerably exceeding the experimental error range.

4.3. Cation specifity

Numerous investigations have shown that neutral salts may exhibit ion specific effects on the thermodynamic and kinetic properties of macromolecule solutions whose magnitude and direction are often describable by the so-called lyotropic or Hofmeister ion series [36]. Correspondingly, a more detailed consideration of the results given in fig. 4 reveals a specific effect of the BSA on γ^*/γ^0, related to the alkali ion species which is most expressed for LiCl.

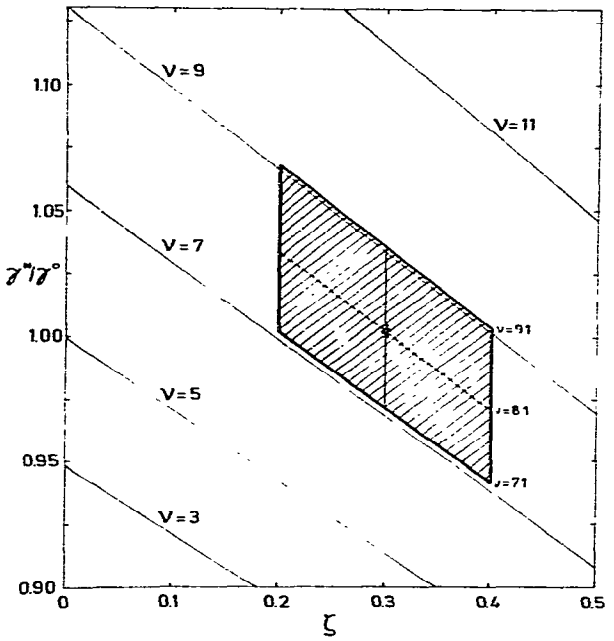

Fig. 5. Influence of the Cl^- ion binding and "non-solvent" water correction terms k_{Cl} and k_w on the final activity coefficients. γ^*/γ^0 calculated with the experimental value of γ = 0.6887 for 0.1 molal NaCl in isoionic BSA solution at m_p = 4.3073 mmol kg^{-1} versus ζ in gram "non-solvent" water per gram BSA with various values ν for the average number of Cl^- ions bound to BSA molecules. $\dot{\Phi}$: γ^*/γ^0 obtained with ν = 8.1 and ζ = 0.3, as chosen in the discussion together with its experimental error range. Shaded area: uncertainty range of γ^*/γ^0 for assumed uncertainties of ± 1 and ± 0.1 for ν and ζ respectively.

As can be seen from the slopes of the regression lines (see fig. 4 and the numerical values in tables 1 and 2), the influence of the BSA component on γ seems to increase in the order of increasing Stokes radii of the alkali ions in solution which is $Cs^+ = Rb^+ \approx K^+ < Na^+ < Li^+$ [37]. This effect, however, is statistically significant only in the case of LiCl. For NaCl, the effect is possibly concealed by the unusually high error range of the RbCl regression line, indicating undetected error causes in these results. Qualitatively, the same behaviour has been found in isoionic hemoglobin alkali chloride solutions [29].

Different qualitative results have been obtained for (1,1)-potassium salts showing a decreasing influence of the protein on γ with increasing anion radius in hemoglobin and BSA solutions [28,38]. From the quantitative aspect, furthermore, the anion specific effect was much more pronounced and seemed to be properly explained, at least for the BSA solutions, by different extent of the specific anion binding known from the literature [11,13–15]. From these findings one might exclude the explanation that the slight cation specific effect shown in fig. 4 could be due to specific cation binding to BSA, all the more as all experiments have shown that isoionic BSA does not bind specifically univalent cations [11,13].

On the other hand, the observed differences of the γ^* values for the alkali chlorides may be caused by the different influences of the alkali ions on the structure of the solvent [36]. As has been pointed out by Samoilov [39], the Li^+ and the Na^+ ions reduce the translational mobility of the water molecules in their nearest neighbourhood ("positive hydration"), whereas the K^+, Rb^+, and Cs^+ ions enhance the translational mobility of the nearest water molecules ("negative hydration"). It is possible that this alkali specific property affects the amount of water "bound" to the protein molecules, resulting in a small cation specific dependence of γ^*/γ^0 in the direction observed, since in eqs. (9), (11), and (15) a constant value for η has been employed for all alkali chloride-BSA solutions.

6. Conclusions and biophysical outlook

The results of our investigations presented here show that the chemical potential of alkali chlorides in isoionic BSA solutions are changed by about 10% only, compared to that in pure salt solutions of equal molality, at protein and salt concentrations which correspond to those in the cytoplasm of the living cell.

The experimentally obtained mean activity coefficients as a function of the protein concentration were analysed, using the known data [15] for the specific binding of Cl^- to BSA and referring to the concept of "protein bound non-solvent water". The residual change of the activity coefficient is probably due to electrostatic interactions between the $BSA(Cl^-)_\nu$-polyions and the salt ions in the "free" water, including a small cation specific effect.

Bovine serum albumin cannot be considered as typical for the cell proteins, but experiments with bovine oxyhemoglobin of varying concentration in alkali halide solutions containing the cations Li^+, Na^+, Rb^+, and Cs^+ and the anions F^-, Cl^-, Br^-, I^-, and NO_3^- showed that hemoglobin exhibits essentially the same influence on γ, except the smaller affinity for anions, compared to BSA [26–30].

Hemoglobin is the main protein within the interior of red blood cells, but the red blood cell is highly specialized and the hemoglobin is larger than the typical cell proteins. It was shown [40] that the average molar mass of the intracellular proteins in Escherichia coli is about 24.0 kg mol^{-1} and in HeLa cells 31.7 kg mol^{-1}. Not more than 5 wt% of all cell proteins have a larger molar mass than 80 kg mol^{-1}. The molar mass pattern of HeLa cells is characteristic for a wide variety of vertebrate tissues except highly specialized cells such as erythrocytes.

The values representing the activity coefficients of the salt in the cell interior components – for principal thermodynamic reasons the ions have to be combined to neutral components – are not known. Single ion activity determinations, e.g. K^+, in the cytoplasm of cells by means of ion-specific electrodes are complicated subject to the generally unknown diffusion potential of this electrochemical cell with transference [41,42].

Therefore the activity coefficient of KCl in the supernatant of calf liver cells with most of the cellular proteins was measured according to the method described in this paper [10,31]. The influence of the cell proteins on the activity coefficient of KCl in the supernatant differs only slightly from hemoglobin and BSA. Assuming that during the fractionation procedure of the liver cells the relevant properties of the proteins in the liver cells did not change one may conclude, that the activity coefficient of simple salts in the cell is not very different from the value in simple model systems, one of which is described in this paper.

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