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Activity coefficients of KCl in highly concentrated protein solutions

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Abstract. As a contribution to the understanding of the thermodynamic state of single salts in living systems, the activity coefficients of KCl were determined in concentrated bovine serum albumin (BSA) solutions. The concentration range studied was 0.01 to 0.5 *M* KCl and zero to 18% wt BSA, thus amply covering physiological conditions. The activity coefficients of the salt were measured using the EMF method with ion exchange membrane electrodes. Keeping the salt concentration constant, the activity coefficients of the salt decrease linearly with protein concentration, the effect being more pronounced for low salt content. The maximal deviations of the activity coefficients with respect to those in pure salt solution amount to ca. 40% for 0.01 *M* KCl and 18% wt BSA. The results were interpreted on the assumption of the superposition of three effects i.e. water bound to BSA molecules as "non-solvent" water, specific Cl^- ion binding and the electrostatic interactions of the polyions with the salt ions. In view of the results it can be concluded that only a small portion of simple intracellular ions are bound, based on the assumption that the cytoplasm of living cells may be regarded as a concentrated protein-salt solution.

Key words: Activity coefficients, protein solutions, ion binding, serum albumin

Introduction

The knowledge of the chemical potentials of the components of the cytoplasm is of central importance in the understanting and interpretation of the life processes and structure of cytoplasm. In terms of thermodynamics all cell functions such as metabolic chemical reactions, flux of substances across the cell membrane and maintenance of the membrane potential, depend on the chemical potentials of all components within the cell.

The main portion of the cytoplasm is best described as an aqueous phase containing 10% to 20% of protein by weight and simple electrolytes amounting to 0.1 to 0.3 moles per kg water (Giese 1973). The properties of the components of this aqueous cell phase – water molecules, protein polyions and electrolyte ions – as well as the interactions between them, as measured by their chemical potentials, will be expected to give insight into the structure and functions of cytoplasm. In particular, the chemical potentials of the electrolytes determine the major part of the colligative properties of cytoplasm, and knowledge of these potentials is of critical importance in the interpretation of the transport of materials across the cell membrane as well as the cell membrane potential.

In this paper the assumption is made that the interactions of salts with proteins in the cytoplasm are of the same nature as in concentrated macromolecule-salt solutions of similar composition. The interactions of KCl with Bovine Serum Albumin (BSA) molecules in water solutions covering a wide range of both salt (0.01 to 0.5 *M*) and BSA (2% to 18% wt) concentrations, will be studied. As a measure of the influence of protein on salt ions, the activity coefficients of salt are used in comparison with those in pure salt solutions of the same concentration. The determination of the activity coefficients of the salts was carried out by measuring the electromotive force of a concentration electrochemical cell using ion-exchange membrane electrodes, a method extensively used for the determination of the ionic activities of salts in protein solutions (Carr 1952, 1968; Lewis and Saroff 1957; Carrol et al. 1959; Saroff and Carrol 1962; Saroff and Lewis 1963; Loeb and Saroff 1964; Baker and Saroff 1965; Scatchard et al. 1957, 1959; Pfister 1971; Pfister and Pauly 1972)

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and mixtures of synthetic polyelectrolytes and simple electrolytes (Kwak 1973; Kwak et al. 1975, 1976).

Several studies of the interaction of simple ions with proteins are to be found in the literature (see for example the reviews of Steinhardt and Reynolds 1969; Hinz 1983; Tsien 1983). However, in nearly all measurements, the concentration of macromolecules is so low that an extrapolation to the average composition of the cytoplasm is impossible. Only in recent years have measurements covering the physiological concentration range been carried out (Pfister 1970, 1971; Pfister and Pauly 1972; Tretter 1975; Schmid 1976; Reboiras et al. 1978, 1986; Nossal et al. 1986). They have shown a ca. 10% decrease in the activity coefficient of the salt in comparison with the value in pure salt solution which implies a relatively small influence of the protein on the thermodynamic state of the small ions.

Experimental

Reference solutions

Pure KCl solutions, used as reference solutions, were prepared from reagent grade dried salt (Merck, p.A.) and bidistilled water. The molalities (0.01 to 0.5 moles per kg water) were adjusted to an accuracy of $< \pm 0.1\%$.

Isoionic BSA-salt solutions

To prepare the protein solutions for measurement, it is necessary to remove any electrolytes that may be present. BSA stock solutions (8%–10% wt) were prepared by dissolving the BSA (Serumalbumin vom Rind, Behringwerke AG, Marburg, FRG), in bidistilled water. In order to remove extraneous salts, the solutions were twice passed through an ion-exchange column at 5 °C (Serdolit M-B). The resulting stock solutions were assumed to be salt-free and isoionic, conductivity 7.5 to $10.5\,\Omega^{-1}\,\mathrm{cm}^{-1}$ (Philips Conductivity Meter PW 9501/01) and pH 4.46 to 4.60 (Beckman Expandomatic SS-2) both at 23 °C. BSA solutions of higher concentrations were produced through water evaporation in a vacuum system at 5 °C. Their concentrations were determined by drying at 110 °C to a constant weight. For the calculations of the protein molality a molecular weight of 66.300 kg per mole was used (Peters 1975, 1985). The final potassium chloride concentration was adjusted by adding the appropriate amount of dried salt. The solutions were stored at 3 °C and used within 2 days.

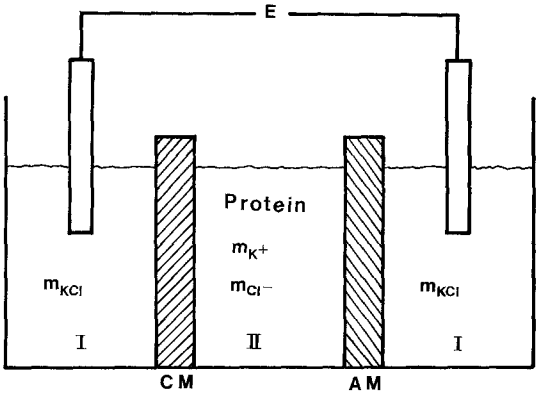

Fig. 1. Three compartment electrochemical cell used for the determination of the mean activity coefficients of KCl in concentrated BSA solutions. *AM, CM*: anion and cation exchange membranes. See text for more details

Procedure

Figure 1 shows the electrochemical cell used. It consists of the protein solution (II) containing m_{K^+} moles K^+ ions and m_{Cl^-} moles Cl^- ions per kg H_2O, separated from two pure solutions (I) of identical concentration, m_{KCl}, by a cation exchange membrane, CM, and by an anion exchange membrane, AM (Nepton AR-111 and Cr-61, Ionics, Watertown, Mass. USA). The cell was constructed in lucite and equipped with an external mechanical stirring system (see Pfister (1971) and Reboiras et al. (1978)). The outer solutions (I) were connected to two identical Cl^--reversible electrodes through salt bridges (KCl saturated). The whole system was installed in a Faraday cage. All measurements were carried out at 23 ± 1 °C. The electromotive force was measured using an X-Y Recorder (Hewlett-Packard mod. 7047A) with an accuracy of ± 0.1 mV. Constant potential readings were obtained within 2 min for dilute protein-salt solutions. For concentrated solutions, however, a longer time was necessary to achieve stable potentials; in such cases, the time needed for reaching the equilibrium was reduced by repeated renewal of the solutions.

The EMF can be related to the chemical potential, and consequently to the activity, of KCl in the protein solution and in the reference solution by considering that, in a reversible isothermal process, the work carried out by the electrochemical cell is given by the difference between the chemical potential of KCl in the protein and reference solution since the contributions at the salt bridges and at the electrodes cancel each other out. This work must be equal to the work done in the external part of the electrical circuit which is $-FE$. Thus we arrive at the relation

$$-FE = \mu''_{KCl} - \mu'_{KCl} = 2RT \ln \frac{(a''_{\pm})_{KCl}}{(a'_{\pm})_{KCl}}. \qquad (1)$$

The EMF is zero when the activities of the KCl salt in the middle and outer compartments are the same. This conclusion is also valid when the membranes do not behave ideally. In this case it can be written, according to Scatchard (1953), Sollner (1968) and Pfister (1971):

$$E = \frac{2RT}{F}(1 - t_{Cl^-}^{CM} - t_{K^+}^{AM}) \ln \frac{(a'_{\pm})_{KCl}}{(a''_{\pm})_{KCl}}, \quad (2)$$

where E stands for the EMF, F the Faraday constant, R the universal gas constant, T the absolute temperature and $a'_{\pm}$ and $a''_{\pm}$ the mean ionic activity of the neutral salt in compartments I and II. t_{K^+} and t_{Cl^-} designate the co-ion transference numbers over the membranes.

If $E = 0$, one obtains:

$$(a'_{\pm})_{E=0} = a''_{\pm}. \quad (3)$$

With

$$a_{\pm}^2 = \gamma_{\pm}^2 m_+ \cdot m_-, \quad (4)$$

where m represents the molality of the ions one obtains

$$\gamma_{\pm} = \frac{(a'_{\pm})_{E=0}}{m_{\pm}} \quad (5)$$

for the stoichiometric mean ionic activity coefficient, $\gamma_{\pm}$, of the neutral salt KCl in the BSA solution.

By changing the concentration and, consequently, the activity of the outer reference solutions and keeping the protein-salt solution unchanged, one obtains EMF-values linearly related to the logarithm of the salt activity of the reference solution as long as the variation of the salt concentration is not so large as to result in a significant change in the co-ion transference numbers in the membranes. The slope of E as a function of $\log(a'_{\pm})$ is also influenced by the stirring velocity in the compartments because of film effects but the value of $(a'_{\pm})_{E=0}$ is not, in agreement with other data (Hale and Govindan 1969; Pfister and Pauly 1969; Pfister 1971).

By linear interpolation to $E = 0$, $(a'_{\pm})_{E=0}$ is obtained. According to Eq. (3) this equals the mean activity, $a''_{\pm}$, in the protein-salt solution. The $a'_{\pm}$ values were calculated from the molalities and the activity coefficient values for pure salt solutions given in the literature (Parsons 1959). The slopes and intercepts of the straight lines were determined by the method of least squares.

The accuracy of the method was tested using KCl solutions of known molality in the middle compartment. The deviations of the $(a'_{\pm})$ measured values with respect to those tabulated, amount on average to $\pm 0.7\%$, the reproductibility being 0.8%. The slopes of the lines E vs. $\log(a'_{\pm})$ were similar with and without BSA in the middle compartment, for the same titration range. A progressive decrease of the slopes was observed with an increase in concentration of salt, caused by an elevation of the mean co-ion transference numbers $t_{Cl^-}^{CM}$ and $t_{K^+}^{AM}$, in the ion-exchange membranes.

Results

Figure 2 shows the stoichiometric mean activity coefficients, $\gamma_{\pm}$, of KCl in isoionic BSA solutions as a function of the KCl molality for different weight values of protein (2% to 18% wt). For comparison,

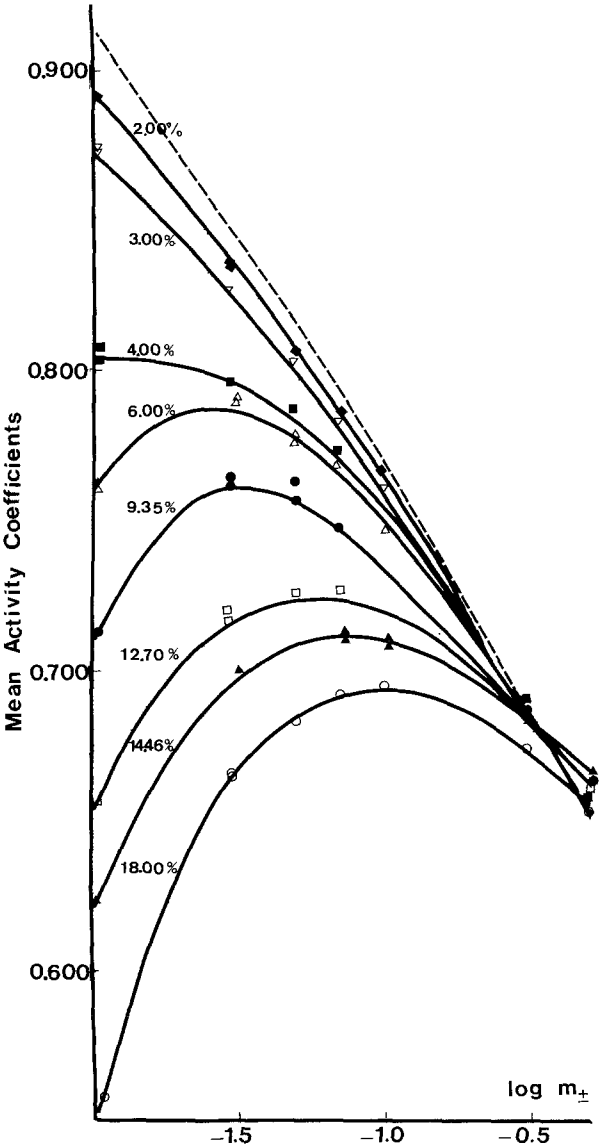

Fig. 2. Stoichiometric mean activity coefficients, $\gamma_{\pm}$, of KCl in isoionic BSA solutions as a function of the molality, for different weight values of the protein, shown by the figure on each curve

the dashed curve gives the mean activity coefficient of KCl in pure KCl solutions. The activity coefficient of the salt is significantly lowered only for salt concentrations less than 0.1 M and high protein concentrations. In the most favourable case, 18% wt BSA and 0.01 M salt, $\gamma_\pm$ is about 38% lower compared with the pure salt solution of the same concentration; at 0.1 moles KCl per kg water, $\gamma_\pm$ is only about 10% less than in pure KCl.

A linear relationship between $\gamma_\pm/\gamma_\pm^0$ and the BSA molality can be obtained from these data, being more marked and showing higher slopes for dilute salt concentrations. A similar linear decrease of $\gamma_\pm$ as a function of protein concentration has been found for the alkali chlorides in the presence of BSA (Pfister and Pauly 1972; Reboiras et al. 1978) and for potassium salts in haemoglobin solutions (Schmid 1976).

Discussion

The term molal activity coefficient only has real meaning if the estimation of the ion concentrations in the protein solution takes into account the ions which are actually free in the solution i.e. which exist in the solution as kinetically independent units and, at the same time, only if the amount of solvent which really is available to them is considered. The term $\gamma_\pm$, as defined by (5), includes the real influence of the protein on the free K^+- and Cl^--ions in addition to all effects caused by the free ions whose concentrations deviate from those measured by the weight of the salt added to the protein solutions.

To attempt to clarify the physical mechanism by which the protein polyions are conditioning the activity coefficient of the salt, we make use of a simple model (Pfister 1971; Pauly 1973) which takes into account the following parameters: *1)* The binding of water on the BSA molecules, whereby the effective KCl molality will increase, assuming that this water is no longer available as solvent (Steinhardt and Reynolds 1969) but while because of this, the activity coefficient must decrease, this mechanism cannot be the only effective one. *2)* The influence of specific Cl^--binding on the BSA molecule, as has been pointed out by several authors (Carr 1968; Scatchard et al. 1950a, b; Steinhardt and Reynolds 1969) and *3)* The electrostatic effect of the BSA molecule on the small ions which is exclusively caused by the bound Cl^- ions, since in aqueous isoionic solutions the BSA molecule may be regarded as a compact molecule carrying practically no net electrical charge in the absence of salts (Peters 1975).

Thus, $\gamma_\pm$ may be separated into three factors although we are aware that each parameter may not be completely independent of the others,

$$\gamma_\pm = \gamma_w \cdot \gamma_v \cdot \gamma_{el}\,, \tag{6}$$

where γ_w, takes into consideration the influence of the water bound to the BSA molecule; γ_v, the influence of Cl^--ion binding on the BSA molecule, and γ_{el}, the influence of the electrostatic field of the charges at the BSA molecule, on the small ions.

In the following we are going to consider these three factors in a qualitative and quantitative way.

The water dipoles interact with the positive and negative electrically charged groups on the protein-polyions and also with some non polar groups on its surface. Whatever the forms in which water may be present (Grigera and Mascarenhas 1978), it is assumed that bound water will act as a "non-solvent" as it does not contain any small hydrated ions. Therefore the amount of KCl added to the BSA solution must be referred to the remaining "free water" in order to obtain the effective molality of the KCl solutions, by means of the equation:

$$m^w = \frac{m}{(1-\eta\, m_{BSA})}\,, \tag{7}$$

where m^w is now the ion concentration in mol per kg total free water, η is the mean amount of water bound in kilogram per mol BSA and m_{BSA} the concentration of BSA in mol per kg water.

Combining Eqs. (7) and (5), we obtain

$$\gamma_\pm = \frac{(a'_\pm)_{E=0}}{m'_{KCl}} \cdot \frac{1}{(1-\eta\, m_{BSA})} \tag{8}$$

and comparing Eqs. (5) and (6), we can write for γ_w:

$$\gamma_w = \frac{1}{(1-\eta\, m_{BSA})}\,. \tag{9}$$

While the remaining expression in (8) contains the product of the parameters $\gamma_\pm$ and γ_{el}.

Many experiments have been reported of measurements of different properties of protein solutions, which have led to the result that approximately 0.2–0.5 g of water per gram of dry protein is bound in mixtures of protein and water (Amberg 1957; Cox and Schumaker 1961; Rosen 1963; Bull and Breese 1968; Hasl and Pauly 1973). In the following a constant value of $\eta = 19.86$ kg non-solvent water per mole BSA has been taken, irrespective of the BSA molality, which corresponds to a value of 0.3 g non-solvent water per g dry BSA.

Consequently γ_v and γ_{el} remain as a measure of the direct influence of the BSA molecule on the salt ions and the salt ions with each other:

$$\gamma_p = \gamma_{el} \cdot \gamma_v\,. \tag{10}$$

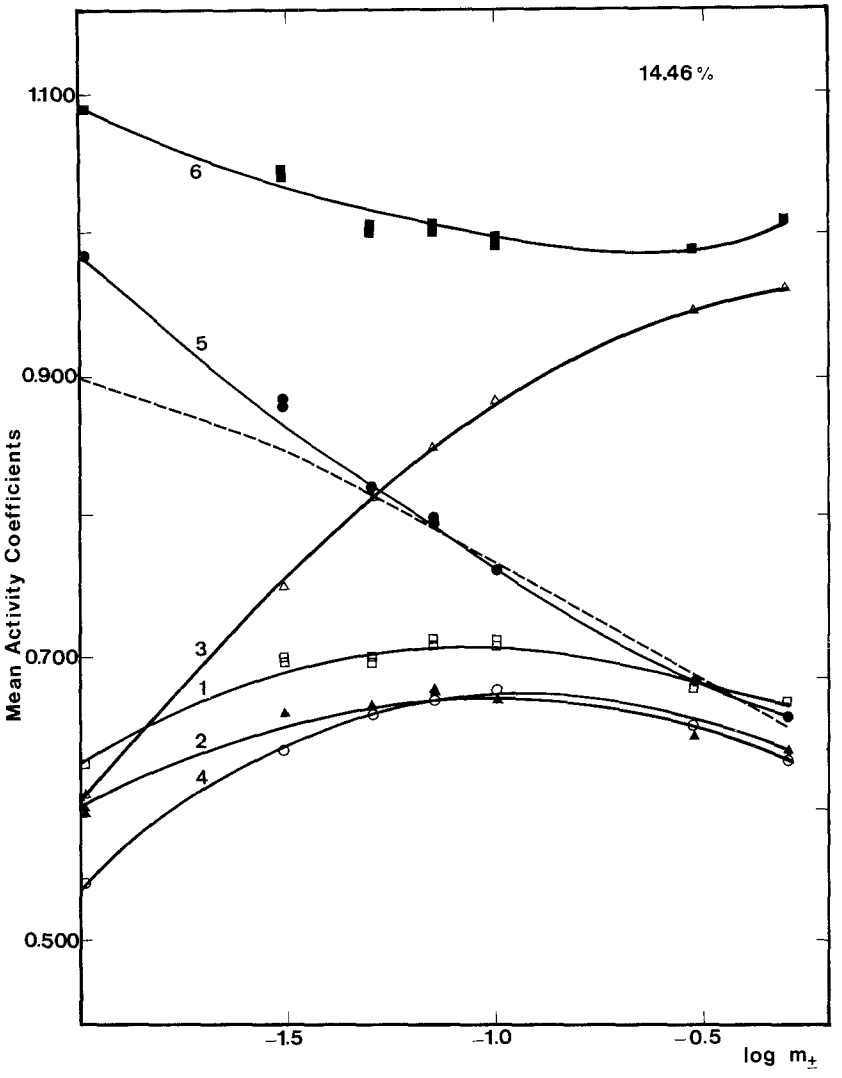

Fig. 3. Mean activity coefficients as a function of the KCl concentration for a 14.46% wt (2.55 mmolar) BSA concentration. With: $\gamma_{\pm}^{0}$, *(dotted line)*, stoichiometric mean activity coefficient of KCl in pure salt solutions; $\gamma_{\pm}$, *(1)* stoichiometric mean activity coefficient in BSA solutions; γ_p, Eq. (10), *(2)* the activity coefficient after taking into account the bound water; γ_v, Eq. (13), *(3)* describes the single influence of the Cl^- bound to the protein; γ^*, Eq. (14), *(4)* the influence of the specific Cl^--ion binding and the interactions between the small ions; γ_{el}, Eq. (15), *(5)* the electrostatic activity coefficient; γ'_{el}, Eq. (16), *(6)* the electrostatic influence of the protein after considering the interactions between the single ions. More details in text

In Fig. 3 the different mean activity coefficients are plotted for a constant protein concentration of 14.46% wt (2.55 mmolar), as a function of the KCl molality. As expected, and this applies to all other BSA concentrations, the consideration of the bound water decreased the activity coefficient with respect to the experimental mean stoichiometric values, shifting the curves to a degree that depends on the protein concentration. As the protein concentration increases, the influence of the bound water on the mean stoichiometric activity coefficient also increases, but to a different degree, depending on the salt concentration as can be seen in Fig. 4, which illustrates the activity coefficients as a function of the BSA molality (constant salt concentration 0.07 *M*). The maximum departure of γ_p from the $\gamma_{\pm}$ values amounts to 8% for 0.5 *M* KCl and 18% wt BSA.

Because of the specific binding of the Cl^--ions to the BSA molecule, the concentration of free Cl^--ions in the KCl-BSA solution will be lower than that added and, according to Eq. (4) a decrease in the mean activity of the salt is expected. The average number of bound Cl^--ions per BSA molecule, ν_{Cl^-}, depends both on the concentration of chloride ions in free water solution and the BSA molality. This may be described by the relation:

$$m^{w,f}_{Cl^-} = m^{w}_{Cl^-} - \nu_{Cl^-}\, m^{w}_{BSA} = m^{w}_{Cl^-}\left[1 - \nu_{Cl^-}\frac{m^{w}_{BSA}}{m^{w}_{Cl^-}}\right], \quad (11)$$

where $m^{w,f}_{Cl^-}$ represents the concentration of free chloride ions per kilogram free water and $m^{w}_{Cl^-}$ the concentration of the total chloride ions per kg free water.

Assuming that no binding of K^+-ions occurs, it follows that $m^{w,f}_{K^+} = m^{w}_{K^+}$. Further, taking into account that $m_{\pm} = (m_{K^+} \cdot m_{Cl^-})^{1/2}$ and $m_{K^+} = m_{Cl^-} = m_{KCl}$, and combining Eqs. (9) and (11), we obtain:

$$\gamma_{\pm} = \frac{(a'_{\pm})_{E=0}}{(m^{w,f}_{K^+} \cdot m^{w,f}_{Cl^-})^{1/2}} \cdot \left[1 - \nu_{Cl^-} \cdot \frac{m^{w}_{BSA}}{m^{w}_{Cl^-}}\right]^{1/2} \cdot \frac{1}{(1 - \eta\, m_{BSA})} \cdot \quad (12)$$

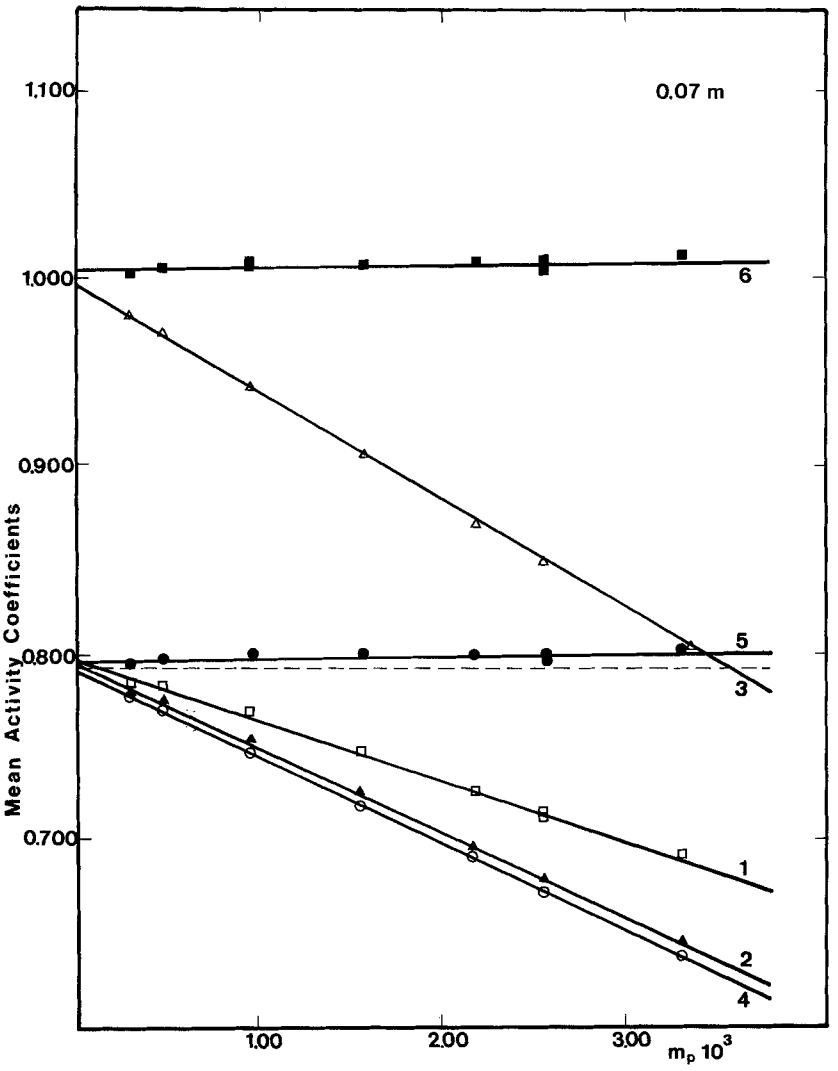

Fig. 4. Mean activity coefficients of KCl as a function of the protein concentration for 0.07 *M* salt concentration. The symbols and notations are the same as those in Fig. 3

Comparing the latter with Eqs. (5) and (6) we can write:

$$\gamma_v = \left[1 - \nu_{Cl^-} \cdot \frac{m_{BSA}}{m_{Cl^-}}\right]^{1/2}. \tag{13}$$

For the calculation of γ_v it is necessary to known ν_{Cl^-} for all solution compositions. According to Scatchard et al. (1950a, b, 1957), ν_{Cl^-} depends both on the free chloride ion concentration in equilibrium with the BSA molecule and the charge of the protein, unless the BSA concentration is kept very low in comparison with the total chloride concentration. The protein concentration range studied in this work is too high to fulfil this condition so we have calculated the ν_{Cl^-} values taking into account the number of different binding sites and their affinity constants on the BSA molecule determined by Scatchard et al. (1957), without ignoring the fact that the use of these constants in our concentration conditions may introduce some degree of uncertainty.

If no electrostatic influence of the protein existed, we could equate γ_{el} to $\gamma^0_{\pm}$ in Eq. (10). The product from γ_v and $\gamma^0_{\pm}$ then describes only the change in the activity coefficient caused by Cl^- binding; furthermore, the comparison with γ_p clearly allows us to show the electrostatic effect caused by the protein. The product of γ_v and $\gamma^0_{\pm}$ will be designed as γ^*:

$$\gamma^* = \gamma_v \gamma^0_{\pm}. \tag{14}$$

Using Eqs. (6), (12) and (13) we can obtain for the electrostatic activity coefficient:

$$\gamma_{el} = \frac{(a'_{\pm})_{E=0}}{m_{KCl}} \cdot \frac{(1 - \eta m_{BSA})}{\left[1 - \nu_{Cl^-} \dfrac{m_{BSA}}{m_{Cl^-}}\right]^{1/2}}, \tag{15}$$

which allows the calculation of γ_{el} from the experimental mean molal activity coefficients. In order to state the electrostatic influence caused by the protein polyion alone, without the mutual interaction of the

small ions, γ_{el} will be devided by $\gamma_{\pm}^{0}$:

$$\gamma'_{el} = \frac{\gamma_{el}}{\gamma_{\pm}^{0}}. \quad (16)$$

The comparison of γ_p with γ^* for constant values of BSA shows a small difference between them. For low protein concentrations (2% and 3% wt) this difference amounts to 5% for 0.01 *M* KCl, changing monotonically as the salt concentration increases. For the 4% and 6% wt BSA concentrations, both curves overlap throughout the whole range of salt concentration, and again for higher concentrations of protein, significant differences are found of the same order of magnitude as at lower concentrations. From these results it is evident that the electrostatic influence of the protein is very low in comparison with the mutual interaction of the small ions (see also both curves on Fig. 4). This is also obvious if we compare the $\gamma_{\pm}^{0}$ curves (dotted lines) with γ_{el}. Only for the lines of the lowest salt concentrations (0.01 and 0.03 *M*) can a significant difference at higher values of protein concentration be observed. For salt concentrations lower than 0.1 *M*, the electrostatic influence of the protein is slightly higher than the interaction of the small ions, while from 0.1 *M* KCl up, the reverse occurs and, at the same time, the values remain constant on further increase of the salt concentration. This is clearly shown when we consider γ'_{el} which, after taking into account $\gamma_{\pm}^{0}$, represents the quantitative percentage value for the lowering of the activity coefficients by the electrostatic influence of the protein. Similar qualitative results with little quantitative differences, have been obtained by Tretter (1975) for the activity coefficients of NaCl in concentrated haemoglobin solutions.

The relatively strong decrease of the mean stoichiometric activity coefficients in the salt concentration range 0.01 to 0.07 *M* KCl is caused almost exclusively by Cl^- binding, however on further increase of the salt concentration the electrostatic effect remains constant after about 0.1 *M* salt concentration, despite the increase in the number of Cl^- ions specifically bound to the protein molecule. The reason for this behaviour could be the secondary effect caused by the shielding of the counter-ion atmosphere around the polyion (Oosawa et al. 1954; Lyons and Kotin 1965). It is a question of the coupling of the two kinds of effects mediated by the salt concentration: on one hand the electrostatic influence of the polyion should increase with the increase in the number of charges on the polyion. However, at the same time, as the salt concentration increases, the opposite effect of screening by the counter ions also increases. As a consequence the electrostatic effect remains constant at salt concentrations greater than 0.1 *M*.

There is a fundamental difference of the salt interactions, as measured by their chemical potentials, with the protein in the lower (2% and 3% wt) and higher concentration ranges. For the two lowest protein concentrations, a small decrease in the activity coefficients of the salt is observed relative to their corresponding values in electrolyte solution without protein. In the whole range of salt concentration the course of the curves is parallel to the curve of the activity coefficients in the pure salt solutions, which necessarily means that the ion-ion interactions are proportional to the square root of the ionic strength, I. However, for higher protein concentrations the shape of the activity coefficient curves changes completely, increasing with the ionic strength at low I values and, after reaching a maximum, descending rather steeply as I increases still further. Between these two sharply different shapes at the lowest and highest protein concentrations, a "transition concentrations" around 4%–6% wt BSA seems to occur. It is only at these protein concentration values that the γ_{el} and $\gamma_{\pm}^{0}$ curves practically overlap each other along the whole range of salt concentrations. From these facts it turns out that other factors, such as short distance interactions between the polyions, which are relatively insignificant at low protein and ionic strength, must come into play at very high values of both parameters, causing a drastic change of several physical properties.

Concluding remarks

In view of the results presented here it is obvious that much more systematic investigations are needed before all the consequences of the concentration effects can be known in detail. Other factors such as the dehydratation effect of different ions must be of central importance to the intermolecular interactions at high concentrations of the polyions. So far we can conclude that only a small portion of the simple intracellular ions are bound, on the basis of the assumption that the cytoplasm of the living cells may be considered as a concentrated protein salt solution.

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