ELSEVIER

Biophysical Chemistry 117 (2005) 217 – 224

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

Why forces between proteins follow different Hofmeister series for pH above and below pI

M. Boström [a,*], F.W. Tavares [b], S. Finet [c], F. Skouri-Panet [d], A. Tardieu [e], B.W. Ninham [f]

[a] *Department of Physics and Measurement Technology, Linköping University SE-581 83 Linköping, Sweden*
[b] *Escola de Química, Universidade Federal do Rio de Janeiro, Brazil*
[c] *ESRF, BP 220, 38043 Grenoble Cedex, France*
[d] *LMCP, 140 rue de Lourmel, 75015 Paris, France*
[e] *P6-PBSF-IM, Case 29, 7 Quai Saint Bernard, 75252 Paris Cedex 05, France*
[f] *Research School of Physical Sciences and Engineering, Australian National University Canberra, Australia, 0200*

Received 9 March 2005; received in revised form 25 May 2005; accepted 26 May 2005
Available online 15 June 2005

Abstract

The relative effectiveness of different anions in crystallizing proteins follows a reversed Hofmeister sequence for pH<pI and a direct Hofmeister sequence for pH>pI. The phenomenon has been known almost since Hofmeister's original work but it has not been understood. It is here given a theoretical explanation. Classical electrolyte and double layer theory deals only with electrostatic forces acting between ions and proteins. Hydration and hydration interactions are dealt with usually only in terms of assumed hard core models. But there are, at and above biological salt concentrations, other non-electrostatic (NES) ion-specific forces acting that are ignored in such modeling. Such electrodynamic fluctuation forces are also responsible for ion-specific hydration. These missing forces are variously comprehended under familiar but generally unquantified terms, typically, hydration, hydrogen bonding, π-electron–cation interactions, dipole–dipole, dipole-induced dipole and induced dipole-induced dipole forces and so on. The many important body electrodynamic fluctuation force contributions are accessible from extensions of Lifshitz theory from which, with relevant dielectric susceptibility data on solutions as a function of frequency, the forces can be extracted quantitatively, at least in principle. The classical theories of colloid science that miss such contributions do not account for a whole variety of ion-specific phenomena. Numerical results that include these non-electrostatic forces are given here for model calculations of the force between two model charge-regulated hen-egg-white protein surfaces. The surfaces are chosen to carry the same charge groups and charge density as the protein. What emerges is that for pH<pI (where the anions are counter-ions) the repulsive double layer forces increase in the order NaSCN<NaI<NaCl, while at higher pH>pI (where anions are co-ions) the forces increase in the order NaCl<NaI<NaSCN. This is in excellent agreement with both solubility experiments and experiments using SAXS. The results are also consistent with cation effects observed in protein solutions. Our results may provide some insights into a long-standing problem in solution chemistry and biology.

Keywords: Hofmeister sequence; Ion; Protein precipitation; Dispersion potential

1. Introduction

In a series of classic papers in the 1880s Hofmeister first demonstrated that salts with fixed cation, but varying in anion, have different capacities in stabilizing protein suspensions [1–3]. The first experiments were on a dispersion of proteins of whole hen-egg-white. Depending on the anion, different concentrations were required to precipitate a prescribed concentration of the proteins. The salts could be ordered in a sequence of effectiveness, that later seemed to be universal, being the same for a number of colloids. Hofmeister, specific ion, effects are now known to be ubiquitous and turn up frequently in biology, solution and biochemistry. For a long time now, despite the

* Corresponding author.
E-mail address: mabos@ifm.liu.se (M. Boström).

0301-4622/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2005.05.010

tantalizing insights of Collins and Washabaugh [2], their explanation has remained a mystery. Of the thousands of papers devoted to specific ion effects, one other, from Ries-Kautt and Ducruix [4] a century after Hofmeister's, provides another suggestive clue. The authors investigated the relative effectiveness of various ions on the solubility of hen-egg-white lysozyme, at pH 4.5. The experiments revealed that the main causes of the effect could be attributed to the anions but that the effectiveness followed a reversed Hofmeister series: $SCN^- > NO_3^- > Cl^-$. This was interesting since the corresponding original paper of Hofmeister [3] gives exactly the opposite anion sequence. This we term a direct Hofmeister series. Later on, the same group demonstrated a direct order for a protein studied at a pH higher than its pI [4]. The large amount of ovalbumin, an acidic protein, present in hen-egg-white, a posteriori explains the anion sequence initially observed by Hofmeister. Revisiting these results, Tardieu and co-workers used small angle X-ray scattering (SAXS) to investigate systematically the relative effects of different anions on lysozyme interactions at pH 4.5 and also on a variety of other proteins at different pHs. They demonstrated an anion-induced additional attraction that followed the reversed Hofmeister series for proteins at pH below their isoelectric point (e.g. for lysozyme $pI \approx 11.16$). Again at $pH > pI$ the strength of the attraction follows a direct Hofmeister series [5–10]. As argued by Finet et al. [5], these curious results provide a benchmark test for the adequacy of any theoretical model. The reason for this reversal in Hofmeister sequence with pH has not been understood, at least not quantified. We will demonstrate here that the reversal phenomenon can be understood, once the NES potentials acting between ions and protein are treated on the same level as electrostatics in a nonlinear double layer theory.

In general Hofmeister effects refer to the relative effectiveness of either anions or cations, individually or as ion pairs, on a wide range of phenomena. These effects have for instance been observed in experiments as diverse as double layer force measurements [11–13], bubble fusion [14], conformational changes of rhodopsin [15], bacterial cell growth [16], yield stresses in silica suspensions [17], cutting efficiency of DNA by restriction enzymes [18], charge of globular proteins [19], surface tension of electrolytes [20], and the solubility of protein solutions [3,4].

The only ionic characteristics included in textbook descriptions of protein and salt solutions are bulk pH, salt concentration, ionic charges and "hydrated ionic radii." Electrostatic theories based on these parameters do not account for the experimentally observed ion-specificity. Since standard theory failed, it has been customary to invoke various specific "ionic characteristics" such as: "structure breaking"; "structure creating"; "lyotropic"; "kosmotropic"; "chaotropic"; "salting-in"; and "salting-out" [1–3]. These concepts attempt to and do encompass the missing ion solvent interactions qualitatively. But the concepts have turned out to be difficult to quantify. There has still been no quantitative explanation of the origin of either the direct or reversed Hofmeister series for different pH. (We remark at this point that the distinction between the theory to be developed below and the customary more qualitative language involving "salting-out' and salting-in" concepts is apparent rather than real. It will be discussed further in our conclusion).

It has recently become clear that the NES forces [1,3], among which are those due to the ion-specific polarizability of ions, neglected in previous theories, play an essential role in determining molecular forces above and beyond electrostatics, which is non-specific. Polarizability is a quantity that measures the response of a particle to a perturbing electromagnetic field. While two charged particles can interact via electrostatic forces there is also a quantum mechanical attraction, the dispersion force, between two charge neutral particles that is related to the polarizabilities of the two particles. So it is too for ions. Dispersion forces have usually been ignored previously. As have those due, e.g., to permanent dipole and dipole-induced dipole, many body forces included in the NES forces are accessible from Lifshitz theory. Or when they have been considered and dismissed they were included incorrectly, or not at the same level as the other forces. (We emphasise that by the mnemonic "dispersion" or NES ("non-electrostatic forces") we mean the totality of many body electrodynamic fluctuation forces embraced by extensions of Lifshitz theory [1,3].) The extensions of Lifshitz theory do formally include ion-specific hydration or self energy and hydration interactions [20]. While electrostatic forces often dominate at low salt concentrations, NES forces become important at high salt concentrations where the electrostatic forces are weakened due to screening. We have shown in previous papers that dispersion forces between an ion and interface often dominate over electrostatic forces above and around physiological salt concentrations [1,3,12,13,20–23].

Our program then is to use these NES forces to explain the direct and reversed Hofmeister series. This is done for pH regulation in buffer and protein solutions in another paper using a spherical cell model [24]. Our objective is to analyze the interaction forces between proteins and other macroions. To do so, we develop the necessary theoretical foundations in Section 2. We present a brief overview of some observed Hofmeister sequences in Section 3. The theory is then used in Section 4 to evaluate the pressure between two model charge-regulated surfaces that carry the same charge group and charge density as the lysozyme. The double layer repulsion decreases when salt concentration increases. We will demonstrate that for $pH < pI$ (where the anions are counter-ions) the double layer repulsion increases in the order $NaSCN < NaI < NaCl$. At higher $pH > pI$ (where the anions are co-ions) the double layer repulsion increases in the order $NaCl < NaI < NaSCN$. This is in excellent agreement with both solubility experiments [4] and second virial coefficients [5–10] deduced from experiments using SAXS. In fact, for large polarizable anions, such as

thiocyanate, the double layer pressure can even become attractive at small protein separations (especially at low pH where the anions are counter-ions). This is consistent with the observation that a much lower concentration of NaSCN is required at pH 4.5 to precipitate lysozyme compared to when NaCl is added [4]. We present a short summary in Section 5. The results provide a strong hint that we may have found further insights into an important long standing question in solution chemistry and biology.

2. Theoretical modeling of ion-specific double layer forces

We wish to draw out and illustrate the essential features of ion-specific double layer forces. To do so we use an ion-specific Poisson–Boltzmann equation. The mean field ionic distribution function has contributions from the non-electrostatic ionic dispersion potentials acting on ions. This will be used to investigate the force between two charge-regulated planar plates. The model plates carry the same charge groups and charge densities as hen-egg-white lysozyme. The calculations will be done for different salts, pH, and plate separations.

In a subsequent publication we demonstrate that there are no conceptually relevant differences between the results that we obtain with this model and corresponding results found from molecular simulations for the potential of mean-force between two charged globular proteins.

We do not include the direct component of the Lifshitz–van der Waals interaction between the two charged plates across an aqueous solution. Assuming that we know the correct ion distributions (we will describe how these are derived later in this section) the double layer pressure between two planar plates a distance L apart can be written as [25,26],

$$P = kT\sum_i \left[c_i(L/2) - c_{0,i}\right] - 2\sum_i \int_{x_0}^{L/2} c_i \frac{dU_i}{dL}dx, \quad (1)$$

where k, T, $c_{0,i}$, $c_i(L/2)$, x_0=2Å, and U_i are Boltzmann's constant, temperature, ion density in bulk solution, ion density at the midplane between the two surfaces, ion size (which is the closest distance the ions can come to the interface), and the ionic dispersion potential acting between each ion and the two interacting proteins. Results have been presented in the past [12,25] that demonstrate that the presence of attractive dispersion potentials acting between ions and interacting charged surfaces asymptotically (at large distances) increase the repulsive double layer pressure. This is partly due to the direct interaction between ions and the surfaces [25]. However, for the intermediate protein separations (a few angstrom up to several tens of angstrom), which is the relevant length scale for protein precipitation, a more complex and interesting picture emerges. The presence of attractive ionic dispersion potentials can either increase or reduce the double layer pressure depending on the charge of the surfaces (i.e. depending on pH and pI). This is related to the nonlinear coupling of NES and electrostatic forces acting on the ions that fundamentally alters the ion distributions.

The ion distributions are obtained, exactly as in our previous papers [12,20,21,27], by solving the nonlinear Poisson–Boltzmann equation for charge-regulated surfaces. This is:

$$\frac{d^2\phi}{dx^2} = -\frac{ec_0}{\varepsilon_w\varepsilon_0}\left(\exp[-(e\phi + U_+(x))/kT] - \exp[(e\phi - U_-(x))/kT]\right), \quad (2)$$

$$\left.\frac{d\phi}{dx}\right|_{x=x_0} = -\frac{e}{\varepsilon_0\varepsilon_w 4\pi r_p^2}\left(\sum_{\text{basic}} \frac{10^{-\text{ph}}\exp[-e\phi/kT]}{K_a^i + 10^{-\text{ph}}\exp[-e\phi/kT]} - \sum_{\text{acid}} \frac{K_a^i}{K_a^i + 10^{-\text{ph}}\exp[-e\phi/kT]}\right), \quad (3)$$

$$\left.\frac{d\phi}{dx}\right|_{x=L/2} = 0. \quad (4)$$

Here ε_w is the dielectric constant of water, ϕ is the self-consistent electric potential, $U_\pm$ are the ionic dispersion potential acting between each ion and the two surfaces, and the pK_a values of lysozyme are given by us elsewhere [21]. We have here normalized the charge density at the planar surface with the surface area of a lysozyme protein (the radius $r_p \approx 16.5$ Å) to ensure that each surface for a specific pH, salt concentration, and ionic species carries the same charge density as the real egg-white lysozyme protein. When the surfaces are close together it is possible to estimate the force between two globular proteins from the corresponding interaction between the two planar surfaces using the Deryaguin approximation. We stress that it is the quantity $10^{-\text{pH}}\exp[-e\phi/\text{kT}]$ that modulates the charge of each acid or basic charge group rather than $10^{-\text{pH}}$ in the boundary condition at the protein surface (Eq. (3)). This is because it is the local electrochemical potential near the protein that regulates the ionizable surface group dissociation rather than the chemical potential of the bulk [21,27,28].

We use here an approximate expression for the dispersion potential acting between an ion and a surface [1,12],

$$U_\pm = B_\pm\left(\frac{1}{x^3} + \frac{1}{(L-x)^3}\right), \quad (5)$$

where the dispersion coefficient ($B_\pm$) for different combinations of ion and protein can be calculated from the frequency-dependent ionic excess polarizability (this is the difference in polarizability compared to the surrounding water) and the dielectric functions of water and of the protein. For sodium, chloride, iodide, and thiocyanate the following values have been used [13,21]: -0.454×10^{-50};

-3.574×10^{-50}; -5.71×10^{-50}; and -10×10^{-50} J m^3. As has been discussed in the literature [23] it would be desirable to have a better characterization of the ionic excess polarizabilities and the dielectric properties of membranes and proteins. But although the values presented may deviate slightly from the correct values for a specific ion and a specific protein they are of the right order of magnitude. Our results are quite general since many proteins (such as lysozyme [21], cytochrome *c* [22], γ- and α-crystallins [5]) should all have very similar dielectric properties in the visible and UV frequency range. We remark that the values used for the ionic excess polarizabilities give consistency between theory and experiment for: surface tension changes and surface potentials in electrolytes [20]; salt dependence of protein charge [21]; activity coefficients of electrolytes [3]; and as we will demonstrate for the reversed and direct Hofmeister sequences.

3. Overview of some experimental results on direct and reversed Hofmeister series

The first demonstration that the protein interactions in solution, as observed with small angle X-ray scattering, vary not only with salt concentration but, at constant ionic strength, with salt type, was done with lysozyme [6,7]. It was shown that, at pH 4.5, i.e. at a pH lower than lysozyme pI (about 11), the efficiency of monovalent anions in inducing an additional attraction followed the reverse order of the Hofmeister series as in Fig. 1. Only minor effects of monovalent cations, checked with chloride salts, could be demonstrated under equivalent conditions.

The salt effect at a pH higher than the pI could not be studied with lysozyme since the pI is too high. Instead, a series of proteins of different sizes, compactness and isoelectric point were analyzed, different γ-crystallins

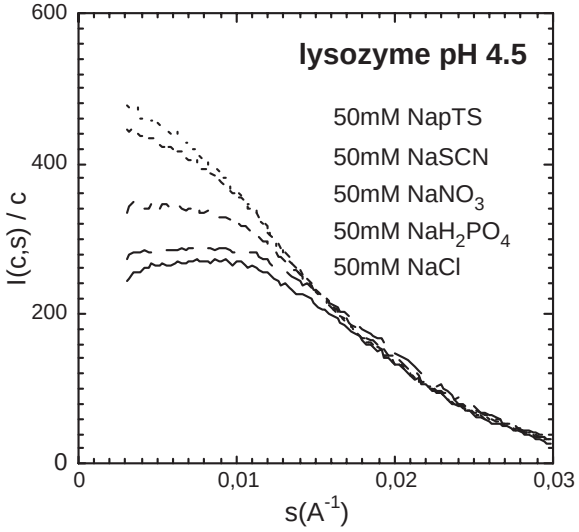

Fig. 1. X-ray scattering curves have been recorded at pH 4.5 in a 100 mg/ml lysozyme solution with different background salt solutions.

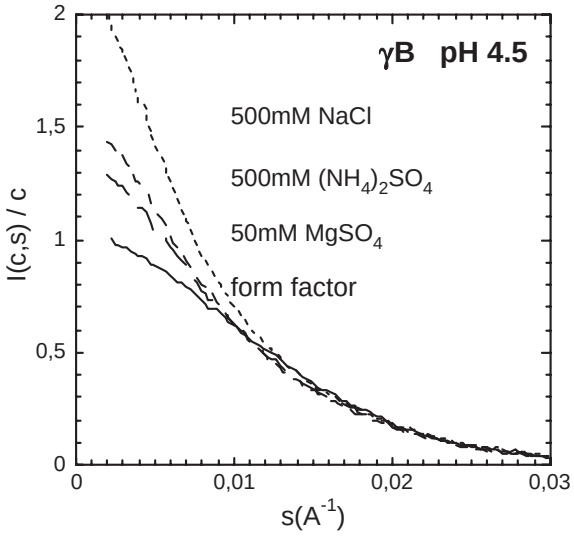

Fig. 2. The γB-crystallin form factor has been recorded using X-ray scattering at pH 4.5 and low protein concentration (6.25 mg/ml). The salt series have been recorded with X-ray scattering with 50 mg/ml γB-crystallin solutions.

(21 kDa, pI ≈ 7 [9]), ATCase (350 kDa, pI ≈ 5 [10]), α-crystallin (800 kDa, pI ≈ 4.5 [9]), and Brome Mosaic Virus (4600 kDa, pI > 7 [8]). In all cases the measured protein interactions were found to become less repulsive upon addition of salt and, with proteins of low molecular weight (≈ 15–20 kDa), to even turn attractive. With monovalent ions, the additional attraction was substantially associated with anions and was seen to induce effects in line with reverse or direct order Hofmeister series depending on whether the proteins were studied at a pH lower or higher than the pI [5]. The reversal of the order could be shown on the same protein with the γB-crystallin, as can be seen in Figs. 2 and 3. Large differences were observed with cations

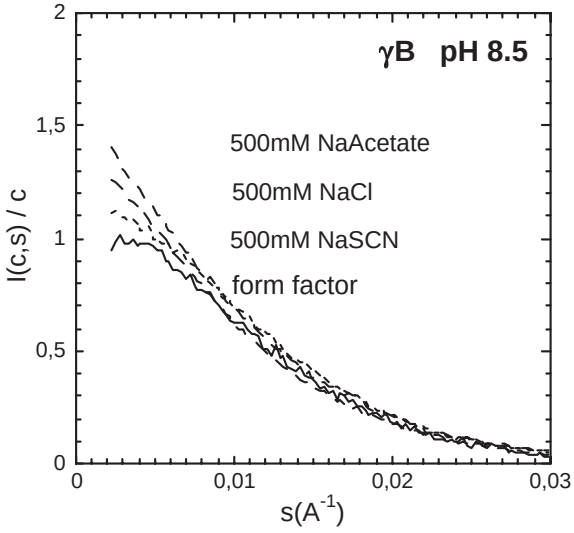

Fig. 3. The γB-crystallin form factor has been recorded at pH 8.5 and low protein concentration (6.25 mg/ml). The salt series has been recorded with X-ray scattering with 25 mg/ml γB-crystallin solutions.

only when passing from monovalent to divalent salts (Fig. 2). The data could be interpreted in terms of an additional short-range attractive potential that increased with the protein charge and with decreasing temperature.

4. Numerical results: understanding direct and reversed Hofmeister sequences

We present a few numerical examples that will explain why Hofmeister sequences can be totally different when the ions are counter-ions as compared to when they are co-ions. While counter-ion effects have not been understood in the past, co-ion effects have been usually dismissed. In electrostatic theories co-ions influence matters only via their contribution to the Debye length. This is a small effect especially with negatively charged proteins, e.g. where the highly charged proteins dominate the Debye length. We first show ion distributions for NaCl and NaSCN that will highlight the underlying mechanisms. After that we present a few figures that give the pressure as a function of ionic species, pH, and plate separation.

In Fig. 4 we show the sum of co-ion and counter-ion concentrations (normalized with bulk values) for 0.15 M NaCl and 0.15 M NaSCN at pH 4 and 12. It is clear from this figure that the first term in Eq. (1) for the double layer pressure (which is directly related to the concentration in the midpoint) gives rise to a reverse Hofmeister sequence at pH 4 and a direct Hofmeister sequence at pH 12. Below the isoelectric point there is strong counter-ion adsorption of SCN^-. This leads to a reduction in the total number of ions in the midplane between the plates and a reduced repulsion, as compared to the corresponding case for Cl^-. Above the isoelectric point there can be co-ion adsorption with SCN^-. This leads to a higher concentration of counter-ions (Na^+)

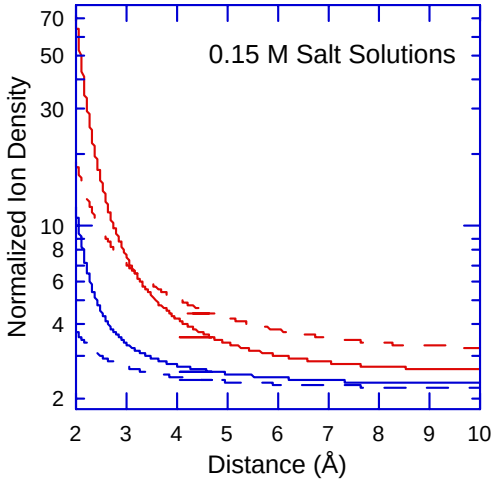

Fig. 4. The ion density (anion+cation) normalized with the bulk values in between two charge-regulated plates (that carry the same charge density and charge groups as hen-egg-white lysozyme) 20 Å apart. The upper (lower) two curves are for pH 4 (pH 12) and the solid (dashed) lines correspond to 0.15 M NaSCN (0.15 M NaCl).

between the two surfaces. While this co-ion adsorption leads to slightly less co-ions with thiocyanate as compared to with chloride, the dominant effect is that it increases the number of counter-ions between the plates. There is a strong clue then that suggests that this is the main source of the different Hofmeister sequences observed when pH is below or above the isoelectric point.

The second term in Eq. (1) for the double layer pressure is related to the ionic dispersion interaction between the ions and the two plates. For attractive dispersion potentials this term gives an attraction. At least in the examples considered here, it increases with the magnitude of the dispersion potential. This is irrespective of pH. The two terms together give rise to a situation where the total pressure due to the salt follows a reverse Hofmeister sequence for pH<pI and a direct Hofmeister sequence for pH>pI.

We are now ready to look in some detail at how the pressure for a fixed plate separation (20 Å) changes with pH as we cross the isoelectric point. The calculation was done for lysozyme (pI ≈ 11.16) for different 0.15 M salt solutions. However the qualitative results would be valid for other proteins as well. In excellent agreement with experiments on a variety of proteins (with lysozyme the experiments could not be done above pI) we see in Fig. 5 that we obtain a reverse Hofmeister sequence for pH<pI and a direct Hofmeister sequence for pH>pI. Close to the isoelectric point where electrostatic effects are very small one can even have attraction with very polarizable anions such as thiocyanate.

It is now possible to understand why hen-egg-white lysozyme and similar proteins at pH below their isoelectric point precipitate at a much lower concentration of NaSCN than with NaCl. It appears that the experimental results obtained for protein solubility [3,4] and small angle X-ray diffraction [5–10] below and above the isoelectric point do make sense.

It is possible to extract some other interesting information from Fig. 5. The pH values at which the pressures in the absence of ionic dispersion potentials are at a minimum should correspond to the point with smallest protein charge, i.e. the isoelectric point (exactly at the pH where there is no protein charge there is obviously no double layer force at all in the absence of ionic dispersion potentials). One could very easily get the incorrect impression from this figure that the isoelectric point drifts towards lower pH for anions when the attractive ionic dispersion potential acts between anion and the protein. However, this is not so. The observed shift in the pressure minima is a delicate balance between charge and ion-specific ion distributions. In fact the real isoelectric point moves towards higher pH as the polarizability of the anions increase. The reason for this is simply that more polarizable anions, such as thiocyanate, are more attracted to and accumulate to a much higher degree near the protein surface. This leads by electrostatic forces to more hydronium being attracted to the surface (or strictly speaking it increases the electrochemical potential of the hydro-

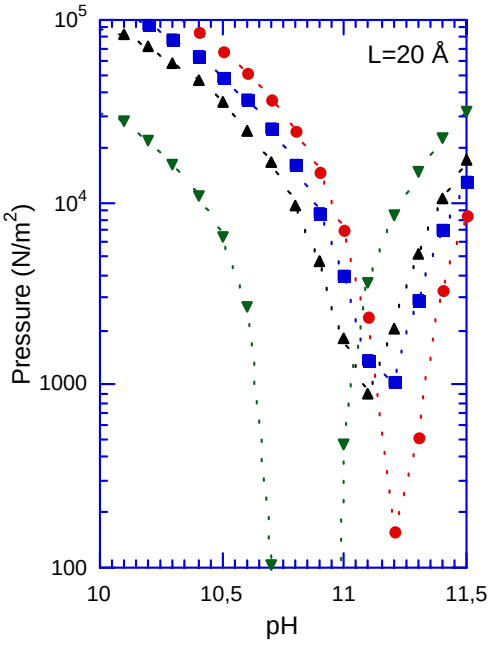

Fig. 5. The double layer pressure in 0.15 M salt solutions between two charge-regulated plates (carrying the same charge density and charge groups as lysozyme) 20 Å apart. We are in all examples excluding the direct van der Waals pressure between the two plates across a water solution. The different curves correspond to: electrostatics (circles); NaCl (squares); NaI (triangles pointing up); and NaSCN (triangles pointing down.) (The lines are only there to aid the eye).

nium ions). That means that for a specific pH, the protein charge will take a slightly higher value in a NaSCN solution than in a NaCl solution. We stress that the effects that we consider here are not due to this weakly ion-specific charge (the influence of ion-specific charges on the pressure is very small). In fact, this effect tends to reduce, or buffer, the effect that we discuss here: both at low pH and at high pH the effect of ion-specific charges give contributions to the pressure that go in exactly the opposite direction compared to the final outcome.

We have also performed calculations (not presented here) for the double layer pressure under the same conditions as those presented in Fig. 5 for 0.15 M KCl (using for K^+ a B value of -1.89×10^{-50} J m^3 [13]) between pH 10 and 12. The observed cation effects were smaller than the anion effects that we have shown here (especially when the cations are co-ions). But again the result is in excellent agreement with experiments. Below the isoelectric point (where the cations are co-ions) the effect when sodium is replaced with potassium is to increase the repulsion and above the isoelectric point (where the cations are counter-ions) the effect is to reduce the repulsion. This apparently explains why Riés-Kautt and Ducruix observed that in a lysozyme solution at pH 4.5 they had to add more KCl than NaCl to crystallize the protein solution.

We also consider the pressure as a function of plate separation for the same systems as in Fig. 5, but for two different pH values. We show the result for a pH below the

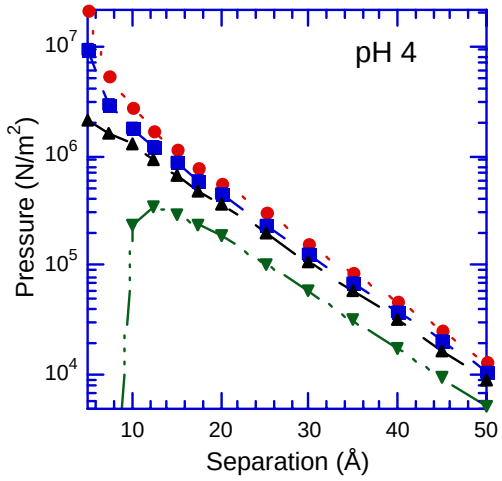

Fig. 6. The double layer pressure as a function of plate separation, at pH 4 for the same salt solutions that was considered in Fig. 5. For small protein–protein separations the force in the presence of NaSCN become attractive and could not be displayed on the logarithmic scale. The pressure becomes at pH<pI less repulsive with increasing ionic polarizability.

isoelectric point (pH 4) in Fig. 6 and for a pH above the isoelectric point (pH 12) in Fig. 7. We can clearly see how the force for all separations relevant for protein precipitation goes in the reversed or direct Hofmeister sequence depending on whether the pH is below or above the isoelectric point.

Notably, these results also seem to be consistent with yield stresses in systems with quite different dielectric properties, e.g. silica suspensions. Franks [17] demonstrated that at higher pH, as the silica nanoparticles became more

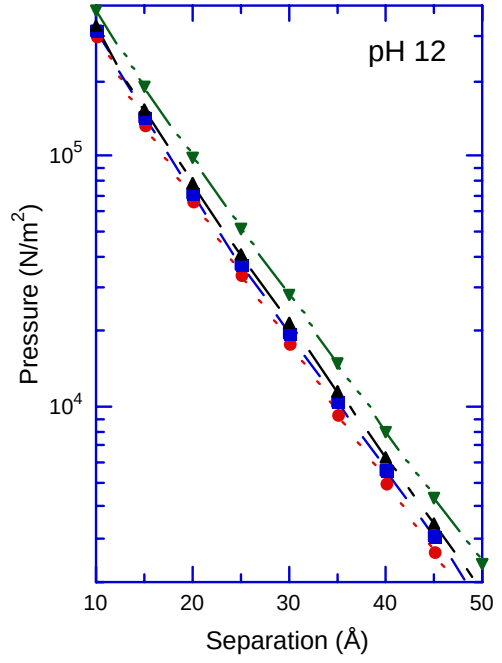

Fig. 7. The double layer pressure as a function of plate separation, at pH 12 for the same salt solutions that was considered in Fig. 5. The pressure becomes at pH>pI more repulsive with increasing ionic polarizability.

negatively charged, the more polarizable cations (counter-ions) gave rise to more attraction between the silica particles than less polarizable cations. At lower pH he found the opposite cation series. This is consistent with the results that we present here for proteins.

While we will not do so here, it is possible to integrate the pressure as a function of distance between two plates to obtain the corresponding force between two spheres (using the Deryaguin approximation). We will come back in a later publication where we will demonstrate that the results that we obtain from Monte Carlo simulations (that considers the proteins as constantly charged spheres), or using the Deryaguin approximation for two spheres, agree well with the results that we present here from double layer theory. The advantage of using double layer theory, besides its appealing simplicity that helps us to focus on the essential points, is that it enables us to consider charge-regulated surfaces.

5. Discussion

While it is clear that ionic NES forces that include ionic polarizabilities and adsorption frequencies have an important role in the experimentally observed Hofmeister series, there are in general also other things that influence ion-specificity. These include, e.g., the ionic size and electron density responsible for the hydration (dispersion self energy and solvation free energy) of ions [29]. At the air–water interface there are also changes in the ionic solvation energies as ions move into the interface region with its inhomogeneous profile of water molecules and dissolved gases [20]). These changes are there necessary to explain the observed electrolyte- specific interfacial tensions. We have here ignored such profiles at a protein–water interface, largely because the protein "surface" is so inhomogeneous, both hydrophobic and hydrophilic, and such profiles in local water structure would be averaged out. For the same reasons we have chosen a fixed cutoff in ion–protein distance of closest approach. Appearances to the contrary, the model DOES include changes in hydration on adsorption, hard and soft ions, through the NES or dispersion B values. More complete values as a function of frequency are obtainable in principle. The general formulae for interaction free energies that include solvation or hydration do go to a constant value at zero distance and our cutoff is sufficiently small to accommodate this [1,3,20].

In order to investigate the further possible role of ion size effects we have also performed Monte Carlo simulations for the force between two globular proteins with constant charges (computational details are the same as in Ref. [13]) that ignored ionic dispersion potentials but included ion size effects (using the ionic radii for Na^+, Cl^-, Br^-, and I^- given by Kunz et al. [3]). We found that ion size effects could not explain the reversal of the Hofmeister sequence. In fact ion size effects gave virtually no effects when the anions were co-ions and reasonably small effects when the anions were counter-ions. The inclusion of ion size furthermore gave an incorrect ion series as compared to experiments.

The main conclusion of the present paper is that the understanding of Hofmeister effects requires that non-electrostatic, electrodynamic ionic forces and electrostatic forces be treated together consistently in a nonlinear theory. When this is done predictive results do emerge.

In a series of papers Tardieu and co-workers used SAXS [5–10] to demonstrate that "the addition of salts not only screens the particle charges, but also induces an additional short range attractive potential, that is a function of the anion type". Irrespective of the macromolecule that they studied, e.g. lysozyme, γ- and α-crystallins, they found that when the macromolecule was studied at a pH lower, or higher, than pI the double layer repulsion followed a reversed, or direct, Hofmeister sequence [5]. In fact, the reversal of the Hofmeister sequence for proteins and other colloids with pH at high salt concentrations has been known experimentally for more than 100 years. For many years it was forgotten and the effects remained an unresolved mystery [30,31]. We have shown in a work parallel to this that pH can change for different salt concentrations and with different anions [24]. However, as far as we can see it is not possible to explain the reversal of the Hofmeister effect discussed here in terms of ion-specific changes in bulk pH. We have presented results that seem to provide the explanation for this perplexing and fundamental problem in solution chemistry and biology. There is no conflict between qualitative notions of salting-out and salting-in effects as further developed by Collins and Washabaugh [2]. The experimentally observed reversed and direct Hofmeister sequences in protein solubility and SAXS come out correctly from theory when the nonlinear coupling of ionic dispersion forces and electrostatic forces are taken into account.

Acknowledgements

M. B. thanks the Swedish Research Council for financially supporting this work. F. W. T. thanks FAPERJ and CNPq (the Brazilian Agencies) for supporting part of this work. We are indebted to Werner Kunz and Didier Touraud for drawing our attention to Refs. [30,31].

References

[1] B.W. Ninham, V. Yaminsky, Ion binding and ion-specificity: the Hofmeister effect and Onsager and Lifshitz theories, Langmuir 13 (1997) 2097–2108.

[2] (a) K.D. Collins, M.W. Washabaugh, The Hofmeister effect and the behavior of water at interfaces, Quart. Rev. Biophys. 18 (1985) 323–422;
(b) K.D. Collins, Ions from the Hofmeister series and osmolytes: effects on proteins in solution and in the crystallization process, Methods 34 (2004) 300–311.

[3] (a) W. Kunz, P. Lo Nostro, B.W. Ninham, The present state of affairs with Hofmeister effects, Curr. Opin. Colloid Interface Sci. 9 (2004) 1–18;
(b) W. Kunz, J. Henle, B.W. Ninham, Zur Lehre von der Wirkung der Salze (About the science of the effect of salts) Franz Hofmeister's historical papers, Curr. Opin. Colloid Interface Sci. 9 (2004) 19–37;
(c) W. Kunz, L. Belloni, O. Bernard, B.W. Ninham, Osmotic coefficients and surface tensions of aqueous electrolyte solutions: role of dispersion forces, J. Phys. Chem., B 108 (2004) 2398–2408 (the entire current opinion issue of Ref. 3 (a) is dedicated to the Hofmeister effect).

[4] (a) M.M. Riés-Kautt, A.F. Ducruix, Relative effectiveness of various ions on solubility and crystal growth of lysozyme, J. Biol. Chem. 264 (1989) 745–748;
(b) C. Carbonnaux, M.M. Riés-Kautt, A. Ducruix, Relative effectiveness of various anions on solubility of acidic hypoderma-lineatum collagenase at pH 7.2, Protein Sci. 5 (1995) 2123–2128.

[5] S. Finet, F. Skouri-Panet, M. Casselyn, F. Bonneté, A. Tardieu, The Hofmeister effect as seen by SAXS in protein solutions, Curr. Opin. Colloid Interface Sci. 9 (2004) 112–116 (and references therein).

[6] A. Ducruix, J.P. Guilloteau, M. Riès-Kautt, A. Tardieu, Protein interactions as seen by solution X-ray scattering prior to crystallogenesis, J. Cryst. Growth 168 (1996) 28–39.

[7] (a) F. Bonneté, S. Finet, A. Tardieu, Second virial coefficient: variations with lysozyme crystallization conditions, J. Cryst. Growth 196 (1999) 403–414;
(b) A. Tardieu, A. Le Verge, M. Malfois, F. Bonneté, S. Finet, M. Riès-Kautt, L. Belloni, Proteins in solution: from X-ray scattered intensities to interaction potentials, J. Cryst. Growth 196 (1999) 193–203.

[8] M. Casselyn, J. Perez, A. Tardieu, P. Vachette, J. Witz, H. Delacroix, Spherical plant viruses: interactions in solution, phase diagrams and crystallization of brome mosaic virus, Acta Crystallogr., D Biol. Crystallogr. 57 (2001) 1799–1812.

[9] S. Finet, D. Vivarès, F. Bonneté, A. Tardieu, Controlling biomolecular crystallization by understanding the distinct effects of PEGs and salts on solubility, Methods Enzymol. 368 (2003) 105–129.

[10] M. Budayova, F. Bonneté, A. Tardieu, P. Vachette, Interactions in solution of a large oligomeric protein P., J. Cryst. Growth 196 (1999) 210–219.

[11] R. Pashley, B.W. Ninham, Double-layer forces in ionic micellar solutions, J. Phys. Chem. 91 (1987) 2902–2904.

[12] M. Boström, D. Williams, B.W. Ninham, Specific ion effects: why DLVO theory fails for biology and colloid systems, Phys. Rev. Lett. 87 (2001) 168103-1-4.

[13] F.W. Tavares, D. Bratko, H.W. Blanch, J.M. Prausnitz, Ion-specific effects in the colloid–colloid or protein–protein potential of mean force: role of salt-macroion van der Waals interactions, J. Phys. Chem., B 208 (2004) 9228–9235.

[14] V. Craig, B.W. Ninham, R. Pashley, Effects of electrolytes on bubble coalescence, Nature 364 (1993) 317–319.

[15] R. Vogel, Influence of salts on rhodopsin photoproduct equilibria and protein stability, Curr. Opin. Colloid Interface Sci. 9 (2004) 133–138.

[16] P. Lo Nostro, A. Lo Nostro, B.W. Ninham, G. Pesavento, L. Fratoni, P. Baglioni, Hofmeister specific ion effects in two biological systems, Curr. Opin. Colloid Interface Sci. 9 (2004) 97–101.

[17] G.J. Franks, Zeta potentials and yield stresses of silica suspensions in concentrated monovalent electrolytes: isoelectric point shift and additional attraction, J. Colloid Int. Sci. 249 (2002) 44–51.

[18] H.-K. Kim, E. Tuite, B. Norden, B.W. Ninham, Co-ion dependence of DNA nuclease activity suggests hydrophobic cavitation as a potential source of activation energy, Eur. Phys. J., E 4 (2001) 411–417.

[19] R.A. Curtis, J. Ulrich, A. Montaser, J.M. Prausnitz, H.W. Blanch, Protein–protein interactions in concentrated electrolyte solutions—Hofmeister-series effects, Biotechnol. Bioeng. 79 (2002) 367–380.

[20] (a) M. Boström, D.R.M. Williams, B.W. Ninham, Surface tension of electrolytes: specific ion effects explained by dispersion forces, Langmuir 17 (2001) 4475–4478;
(b) M. Boström, B.W. Ninham, Energy of an ion crossing a low dielectric membrane: the role of dispersion self-free energy, Biophys. Chem. 114 (2005) 95–101;
(c) M. Boström, B.W. Ninham, Contributions from dispersion and born self-free energies to the solvation energies of salt solutions, J. Phys. Chem., B 108 (2004) 12593–12595;
(d) M. Boström, W. Kunz, B.W. Ninham, Hofmeister effects in surface tension of aqueous electrolyte solution, Langmuir 21 (2005) 2619–2623.

[21] M. Boström, D. Williams, B.W. Ninham, Specific ion effects: why the properties of lysozyme in salt solutions follow a Hofmeister series, Biophys. J. 85 (2003) 686–694.

[22] M. Boström, D.R.M. Williams, B.W. Ninham, Specific ion effects: role of salt and buffer in protonation of cytochrome *c*, Eur. Phys. J., E 13 (2004) 239–245.

[23] M. Boström, V.S.J. Craig, R. Albion, D.R.M. Williams, B.W. Ninham, Hofmeister effects in pH measurements: role of added salt and co-ions, J. Phys. Chem., B 107 (2003) 2875–2878.

[24] (a) M. Boström, B. Lonetti, E. Fratini, P. Baglioni, M.C. Pinna, A. Salis, M. Monduzzi, B.W. Ninham, Why pH titration in buffer and protein solutions follow a Hofmeister series, J. Phys. Chem., B (submitted for publication);
(b) M.C. Pinna, A. Salis, M. Monduzzi, B.W. Ninham, Hofmeister series: the hydrolytic activity of *Aspergillus niger* lipase depends on specific anion effects, J. Phys. Chem., B 109 (2005) 5406–5408.

[25] S. Edwards, D. Williams, Double layers and interparticle forces in colloid science and biology: analytic results for the effect of ionic dispersion forces, Phys. Rev. Lett. 92 (2004) 248303 1–4.

[26] Y. Burak, D. Andelman, Discrete aqueous solvent effects and possible attractive forces, J. Chem. Phys. 114 (2001) 3271–3283.

[27] B.W. Ninham, V.A. Parsegian, Electrostatic potential between surfaces bearing ionizable groups in ionic equilibrium with physiologic saline solution, J. Theor. Biol. 31 (1971) 405–428.

[28] B. Mörnestam, K.-G. Wahlund, B. Jönsson, Potentiometric acid–base titration of a colloidal solution, Anal. Chem. 69 (1997) 5037–5044.

[29] P. Jungwirth, D. Tobias, Molecular structure of salt solutions: a new view of the interface with implications for heterogeneous atmospheric chemistry, J. Phys. Chem., B 105 (2001) 10468.

[30] K.H. Gustavson, Specific ion effects in the behaviour of tanning agents toward collagen treated with neutral salts, in: H. Boyer Weiser (Ed.), Colloid Symposium Monograph, The Chemical Catalog Company Inc., New York, 1926, and references therein.

[31] J. Loeb, The proteins and colloid chemistry, Science LII (1920) 449–456.