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The interfacial tension concept, as revealed by fluctuations

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ABSTRACT

A simple, didactic model that could have conclusively interpreted the complexity of specific salt (Hofmeister-) effects on protein solubility and function, using a single physical quantity as a central parameter, has long been missing. Via surveying a row of recent papers we show in this review that a phenomenological formalism based on the salt-induced change of protein–water interfacial tension ($\Delta\gamma$) is able to account for a wide range of Hofmeister effects, including also such "exceptions", where inverse or "V-shaped" Hofmeister series occurs. A close relationship between protein–water interfacial tension and conformational fluctuations is pinpointed on theoretical grounds, then it is shown how one can use a complex experimental arsenal to demonstrate conformational fluctuations on two prototypical proteins, the membrane protein bacteriorhodopsin and the cytoplasmic protein myoglobin. Finally, via the results of recent and new molecular dynamics simulations on a model peptide, the tryptophan-cage miniprotein, independent evidences are given in favor of the interfacial tension concept, at the same time demonstrating the predictive power of the theory. It is shown that salt-induced fluctuation changes of surface-exposed amino acid groups can be used as a sensitive measure for mapping the local features of Hofmeister effects on protein conformations. General implications of the interfacial tension concept are also discussed.

1. Introduction

Understanding life phenomena based on the principles of physics is the main goal of a biophysicist. As far as biological macromolecules are concerned, e.g., cracking the DNA code, or modelling proteins based on atomic-level physical interactions can be listed among the basic achievements of the discipline. However, the application of less detailed physical models can also be useful to describe some regular patterns appearing at higher levels of hierarchical organization of biological matter, and predict its behavior under special conditions (see, e.g., the electrical conductance-based Hodgkin–Huxley model for the action potential of neurons [1]). Specific salt (Hofmeister-) effects (HE) on protein aggregation and function also seem to belong to this category (for a recent review, see [2]). Despite that their detailed interpretation using the modern arsenal of theoretical physics still represents a major challenge, a phenomenological description based on physical principles appears to be much closer, and it could also be of substantial practical and philosophical value. In this review, after giving a brief historical introduction to the phenomenology of Hofmeister effects, we discuss two major strategies suggested for its formal description: one based on conformational fluctuations, and another one based on the protein–water interfacial tension as a central physical parameter describing a wide variety of Hofmeister effects. It will be shown how the interfacial tension concept is linked to conformational fluctuations via basic theoretical relations such as the Fluctuation–dissipation theorem, and its predictive power will be demonstrated by experimental examples. Finally, molecular dynamics simulations carried out on a model protein will be used to give a microscopic interpretation for the interfacial tension concept, whose general implications will also be discussed.

2. The phenomenology of Hofmeister effects

If we consider protein solubility in a complex environment of water solutions of a few hundred millimolar salt concentrations, and ignore exceptions, its phenomenology is quite simple: salts called kosmotropes decrease solubility ("salting out"), while salts called chaotropes increase it ("salting in") [3,4]. The well-known Setschenow's law gives a simple, quantitative description for the high-concentration limit, supported by a vast number of experimental evidences:

$$\log\frac{S_0}{S} = K_s c_s \tag{1}$$

where S_0 and S are the solubility values of a protein in pure water and in a salt solution of concentration c_s, respectively. The Setchenow-constant (K_s) is positive for kosmotropes and negative for chaotropes [5].

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The formal simplicity of Eq. (1) has long inspired many biophysicists to assign a physical quantity to K_s. One of the most popular approaches taken by such a motivation was the so-called cavity model, suggested originally by Melander and Horváth [6], and modified later by many experts, including most notably in references [7–10]. The main idea of these pioneering studies was that they coupled ion-induced structural changes of water to Hofmeister effects via the air–water surface tension, what was also known to change as a function of salt concentration according to the Hofmeister series. This is described by the Heydweiller equation [11]:

$$\Delta\gamma_a = K_H c \tag{2}$$

where the subscript "a" denotes the air–water interface; the Heydweiller constant, K_H (also called the surface tension increment in the literature) with chaotropes having smaller values than kosmotropes [3]. The free energy change associated to the solvation of a protein molecule was then assumed to be given by a *gedanken experiment* involving the formation of a cavity of the size and shape of the cosolute (e.g., a protein), and subsequently, the transfer of it (from air or vacuum) into this cavity; hence the involvement of air–water surface tension. While this approach worked relatively well for small solutes like benzene that is salted out by practically all Hofmeister salts [10,12], it failed for proteins, giving a qualitative interpretation only for one side of the Hofmeister phenomena, namely, kosmotropic salting out, whereas chaotropic effects remained unexplained in this framework. The formal reason is that all investigated salts actually increased air–water surface tension [3], in other words K_H was always positive, hence, cannot account for the sign change in the Setchenow constant observed when coming from the kosmotropic to the chaotropic side. Therefore, other factors such as a generalized electrostatic interaction between chaotropic ions and the protein [6], or introduction of a compensatory binding term to the usual surface tension increment [8] were suggested to be considered to describe the solubilizing effect of chaotropes. Although such effects might in fact occur, but being specific to the protein–electrolyte interface, they could not be included in the air–water surface tension formalism with reasonable physical meaning. Facing these problems, Baldwin concluded in 1996 that a different mechanism is needed to interpret the ion-specific dependence of protein solubility [10]. Nevertheless, a lot of interesting investigations have been carried out concerning the conceptually and practically simpler air–water interface since then [13–16], but the detailed discussion of related results is beyond the scope of this review.

3. The fluctuation concept

Based on kinetic experiments carried out on the paradigmatic protein bacteriorhodopsin, Dér and coworkers suggested the involvement of salt-induced conformational flexibility, and, closely related to that, fluctuations changes as a new concept in the interpretation of Hofmeister effects [17,18]. Considering protein aggregation as a thermally activated process (Eq. (3)), it was hypothesized by Neagu et al. that addition of kosmotropic and chaotropic salts alters the fluctuation levels of end states and the activation barrier differently [18].

$$P_{aggregate} \underset{k_{12}}{\overset{k_{21}}{\rightleftharpoons}} P_{solution} \tag{3}$$

To establish a quantitative model, an exponentially correlated, Markovian dichotomous noise, $\xi(t)$, of amplitude proportional to the salt concentration, c_s, was added to the free energy levels of the system composed of monomeric and aggregated proteins. The standard free energy of activation in the presence of salts could then be written as $\Delta\tilde{G}_{ij} = \Delta G_{ij} + c_s \cdot a_{ij} \cdot \xi(t)$, that is, the magnitude of energy barrier fluctuations corresponding to the process $j \to i$ is given by $a_{ij} \cdot c_s$. The equilibrium value of the time evolution of the fluctuation-averaged protein concentration yields protein solubility in the presence of noise (salts): $lim\langle c_2\rangle(t) \equiv S$. Further details are given in ref. [18], here we merely state the result:

$$S = S_0 \frac{\cosh\left[\frac{(a_{21}-a_{12})c_s}{RT}\right] + r \cdot \cosh\left(\frac{a_{21}c_s}{RT}\right)}{1 + r \cdot \cosh\left(\frac{a_{12}c_s}{RT}\right)} \tag{4}$$

where $r = \lambda/k_{12}$, λ being the reciprocal noise correlation time. It can be seen that Eq. (4) approaches the Setschenow equation at the high-concentration limit (c_s), if the noise correlation time is low compared to the aggregation time constant ($r \gg 1$):

$$S \cong S_0 \ \exp\left[-\frac{(a_{12}-a_{21})c_s}{RT}\right] \tag{5}$$

Comparison of Eqs. (1) and (5) yields the expression of the Setschenow constant as a function of noise amplitudes:

$$K_s = \frac{a_{12}-a_{21}}{2.303 \cdot RT} \tag{6}$$

i.e., in this representation Hofmeister effects stem from the asymmetry of barrier fluctuations.

The theory was successfully tested by interpreting solubility data of deoxy-HbS [19], and gave the first formalism suitable for a satisfactory description of protein solubility data concerning Hofmeister effects along the whole range of cosolute concentrations. At variance with earlier approaches, based on exponential fits of protein solubility data in some salt concentration intervals [19,20], it also identified the concentration range in which Setschenow's law is expected to be valid, even if experimental data are available only at lower concentrations. Given the general nature of the assumptions used in this theory, it was suggested to analyse other reactions of macromolecules influenced by the presence of cosolutes, e.g., kinetic phenomena associated with Hofmeister effects. On the whole, based on the assumption of salt-induced changes in conformational fluctuations of macromolecules, this theory proved to be rather successful in describing protein solubility data, and gave an independent physical explanation of the Setchenow equation, too. Establishing a thorough experimental and theoretical link of conformational fluctuations to the physics of the protein–water interface, nevertheless, represented a scientific challenge for follow-up studies.

4. The interfacial tension concept (ITC)

In 2007, Dér et al. came out with a concept [21], in which, instead of relying on the cavity model bound to air–water interfacial tension, they interpreted Setschenow's equation directly by protein–water interfacial tension, using a simple thermodynamic treatment.

In order to link solubility with interfacial tension, they considered the chemical equilibrium of a solid (e.g., a protein aggregate) and its solute (individual protein molecules) in pure water. Using the nomenclature of the present study, one can write

$$\mu_s = \mu_0 + RT \ln(x_0) \tag{7}$$

where μ_s and μ_0 are the chemical potentials of the pure solid and solute (the individual, isolated protein molecules in pure water), respectively, and $RT\ln(x_0)$ is the "mixing term" (see, e.g., ref. [22]), with x_0, the mole fraction of the solute in saturated solution (i.e., its solubility) [23]. (Note that the 0 indices here refer to the case of pure water solvent, and not the standard quantities.) For the same solid in another solvent (e.g., an aqueous solution of a Hofmeister salt)

$$\mu_s = \mu + RT \ln(x) \tag{8}$$

follows, where μ and x denote quantities relevant to the other solvent.