Biochimica et Biophysica Acta 1830 (2013) 4564–4572

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Hofmeister ions control protein dynamics

Balázs Szalontai [a], Gergely Nagy [b,c,1], Sashka Krumova [d], Elfrieda Fodor [e], Tibor Páli [a], Stefka G. Taneva [d,2], Győző Garab [f], Judith Peters [c,3], András Dér [a,*]

[a] Institute of Biophysics, Biological Research Centre of the Hungarian Academy of Sciences, H-6726 Szeged, Temesvári krt. 62, Hungary
[b] Wigner Research Centre for Physics, Institute for Solid State Physics and Optics, Hungarian Academy of Sciences, P.O. Box 49, H-1215 Budapest, Hungary
[c] Institut Laue–Langevin, BP 156, 38042 Grenoble Cédex 9, France
[d] Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl.21, 1113 Sofia, Bulgaria
[e] Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, H-6726 Szeged, Temesvári krt. 62, Hungary
[f] Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, H-6726 Szeged, Temesvári krt. 62, Hungary

ARTICLE INFO

Article history:
Received 16 April 2013
Received in revised form 24 May 2013
Accepted 28 May 2013
Available online 7 June 2013

Keywords:
Hofmeister effect
Differential scanning calorimetry
Neutron scattering
Fourier transform infrared spectroscopy
Protein structural fluctuation
Bacteriorhodopsin

ABSTRACT

Background: Recently, we have elaborated a thermodynamic theory that could coherently interpret the diverse effects of Hofmeister ions on proteins, based on a single physical parameter, the protein–water interfacial tension (Dér et al., Journal of Physical Chemistry B. 2007, 111, 5344–5350). This theory, implying a “liquid drop model”, predicts changes in protein conformational fluctuations upon addition of Hofmeister salts (containing either kosmotropic or chaotropic anions) to the medium.
Methods: Here, we report experimental tests of this prediction using a complex approach by applying methods especially suited for the detection of protein fluctuation changes (neutron scattering, micro-calorimetry, and Fourier-transform infrared spectroscopy).
Results: It is demonstrated that Hofmeister salts, via setting the hydrophobic/hydrophilic properties of the protein–water interface, control conformational fluctuations even in the interior of the typical membrane transport protein bacteriorhodopsin, around its temperature-induced, unusual $\alpha(\mathrm{II}) \rightarrow \alpha(\mathrm{I})$ conformational transition between 60 and 90 °C. We found that below this transition kosmotropic ($COOCH_3^-$), while above it chaotropic (ClO_4^-) anions increase structural fluctuations of bR. It was also shown that, in each case, an onset of enhanced equilibrium fluctuations presages this phase transition in the course of the thermotropic response of bR.
Conclusions: These results are in full agreement with the theory, and demonstrate that predictions based on protein–water interfacial tension changes can describe Hofmeister effects and interpret protein dynamics phenomena even in unusual cases.
General significance: This approach is expected to provide a useful guide to understand the principles governing the interplay between protein interfacial properties and conformational dynamics, in general.

1. Introduction

Hofmeister effects (HEs) cover a wide range of salt-induced phenomena on proteins, and on colloidal particles in general, including changes of solubility, denaturation, or enzyme kinetics. In his original work, Hofmeister [1] reported that certain salts (primarily their anions) either decrease or increase protein solubility when present in the solution at moderate or high concentrations (usually above 100 mM), and arranged them into the so-called Hofmeister series (HS) according to the sign and the magnitude of the effect they exerted. The HS of the most important anions, in descending order of their precipitating ability, is as follows: $F^- \approx SO_4^{2-} > HPO_4^{2-} > CH_3COO^- > Cl^- > NO_3^- > Br^- > ClO_3^- > I^- > ClO_4^- > SCN^-$. Members in the series left of the “Hofmeister-neutral” Cl^- are precipitants, while the ones right of it are solubilizers. An impressive number of follow-up studies show that the same HS emerges in other phenomena, like denaturation of proteins, inhibition or activation of enzymes, as well (for reviews, see [2–4]). HEs live their renaissance in the past few years. Besides their significance in colloid chemistry, preparative biochemistry and biotechnology, they have been successfully applied in studying the function and dynamics of macromolecular structures [5,6]. In addition, a recent review calls the attention to their role in basic pathophysiologic issues [7].

---

[*] Corresponding author at: Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, H-6726 Szeged, Temesvári krt. 62., Hungary. Tel.: +36 62 599 606; fax: +36 62 433 133.
E-mail addresses: szalontai.balazs@brc.mta.hu (B. Szalontai), nagy@ill.fr, jpeters@ill.fr (G. Nagy), sakrumo@gmail.com (S. Krumova), fffrida7@gmail.com (E. Fodor), stefka.germanova@ehu.es (S.G. Taneva), der.andras@brc.mta.hu (A. Dér).

[1] Present address: Paul Scherrer Institute, Laboratory for Neutron Scattering, 5232 Villigen PSI, Switzerland.

[2] Present address: Unidad de Biofísica (CSIC-UPV/EHU) and Departamento de Bioquímica y Biología Molecular, Universidad del País Vasco, 48080 Bilbao, Spain.

[3] Present address: University Joseph Fourier, UFR PhITEM, BP 53, 38041 Grenoble Cédex 9, France; Institut de Biologie Structurale, 41 rue Jules Horowitz, 38027 Grenoble Cédex 1, France.

0304-4165/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbagen.2013.05.036

Despite the widespread occurrence of HEs, and the extensive research efforts focused on them, their interpretation has remained a matter of debate [8], because of the lack of a unifying formalism covering the entire spectrum of salts from "salting out" to "salting in" effects, i.e. from precipitants to solubilizers. One approach [9] correlated these attributes with effects on water structure, in particular on the fraction of hydrogen-bonded water molecules: precipitants promote H-bond formation between the water molecules in their vicinity, and are therefore called kosmotropes, while solubilizers break H-bonds between water molecules, and are therefore called chaotropes. In another branch of interpretations, dispersion forces were suspected to be the main factor responsible for HEs [10]. Although these are certainly among the main microphysical factors responsible for the Hofmeister effects, all attempts aiming to assign a single physical parameter as an approximate measure for the whole diversity of HEs, however, have failed for more than a century.

In a recent work, we have demonstrated that a unified, phenomenological formalism is able to qualitatively account for the entire range of HE-related phenomena [11]. The crucial difference between our approach and the previous attempts of similar intention was that, instead of building on the conventionally used air–water interface [9], we took protein–water interfacial tension (γ_{pw}) as the principal physical parameter to describe Hofmeister effects. The most important conclusion of our theory is that HEs are manifested via the surface-dependent term of the free energy ($G_s = \gamma_{pw} \cdot ASA$, where ASA is the solvent accessible surface area) of the proteins (or, in general, of colloid disperse systems) [12]. In other words, they modify the hydrophobic effect at protein–water interfaces [13]. The addition of kosmotropic or chaotropic salts to the solvent increases or decreases the solute–water interfacial tension, respectively, and thus, G_s should increase or decrease accordingly. The alteration of G_s is the driving force behind the observed HEs affecting either the aggregation or the conformation of the proteins. Note that γ_{pw} should, necessarily, depend also on the quality of the solvent-exposed protein surface, and may even take negative values, too (e.g., in case of proteins of naturally open conformation) [11,14].

An implication of our theory is that HEs are manifested by changing transition rates and equilibrium constants in reactions accompanying major conformational changes that involve changes in the water-exposed surface area of macromolecules and supramolecular assemblies. (Such effects have recently been exemplified by a detailed study of Hofmeister effects on the function of photoactive yellow protein [5].) It was also established that interfacial tension and protein stability are interconnected by protein conformational fluctuations, thus providing the keystone for the microscopic interpretation of HEs [11,15]. In consequence of the above view, the same HS should appear for salt-induced protein conformational fluctuations as for precipitation or conformational changes.

In spite of the success of this theory in describing the diverse Hofmeister phenomena, so far direct experimental evidences for salt-induced changes of protein conformational fluctuations have been missing. Hence, here we used a complex experimental approach to monitor temperature-induced changes of protein structure and dynamics, with methods specially adapted for observing changes in conformational fluctuations (neutron scattering, differential scanning calorimetry (DSC), and Fourier transform infrared spectroscopy (FTIR)). Our model object was the prototypical retinal protein, bacteriorhodopsin (bR) from *Halobacterium salinarum* [16]. Also known as the simplest ion pump in biological systems, bR is one of the best-characterized and most robust membrane proteins, subjected to non-specific anion binding effects. Despite, both its structure and function have been shown to be influenced by interactions with Hofmeister anions [11,17]. In the present study, special attention was paid to the reversible conformational change of bR in the course of its heat denaturation, the α(II) → α(I) conformational transition between 60 and 90 °C, where enhanced changes of structural fluctuations were expected to arise [18,19], due to the associated alterations of the solvent-exposed surface.

So far, there have been only a few reports about proteins having an "open" conformation as their naturally most stable form [3], and bR is considered to be such one. Namely, previous FTIR measurements on bR detected α(II) helices below ca. 50 °C [18], and between 60 and 90 °C, bR undergoes an α(II) → α(I) conformational transition [19]. In α(I), however, the helix is known to be more tightly packed than in α(II) [18,20]. It implies that, in contrast to most proteins, here the accessible surface area (ASA) of the protein is higher at lower temperatures (α(II)), and ASA decreases upon the temperature-induced transition of the secondary structure (to α(I)). For such a case, our theory [11] predicts an unusual phenomenon to occur: instead of kosmotropic, here chaotropic salts should have a stabilizing effect; consequently, they are expected to shift the α(II) → α(I) transition to higher temperatures, while kosmotropes are expected to destabilize the open α(II) protein conformation, therefore shifting the transition toward lower temperatures, as it has been indicated by our former DSC experiments [11]. We took advantage of this unusual feature of bR to test the predictive power of our HE-interpreting theory.

2. Materials and methods

2.1. Bacteriorhodopsin-containing purple membrane (bR) isolation

Purple membranes were isolated according to the standard procedure [21]. Purple membranes were always re-suspended in D_2O-based buffers, due to the needs of neutron scattering and FTIR experiments (10 mM HEPES in D_2O at pD 6.6, containing 500 mM kosmotropic, Hofmeister-neutral, or chaotropic salts, $NaCOOCH_3$, NaCl, or $NaClO_4$, respectively, as required). The bR samples, treated with these salts will be denoted as bR-$NaCOOCH_3$, bR-NaCl, and bR-$NaClO_4$ throughout the paper.

2.2. Neutron scattering experiments

For neutron scattering experiments the suspensions of bR-containing purple membranes were centrifuged for 20 min at 40,000 *g*. The pellets were placed in flat rectangular aluminum sample holder with an area ($30 \times 40\ mm^2$) adapted to the dimensions of the incident neutron beam. The sample holders were hermetically closed and used for the experiment.

The experiments were carried out on the IN13 backscattering spectrometer at the Institut Laue Langevin (ILL, France). Two samples were measured by elastic incoherent neutron scattering to determine the atomic mean-square displacements $<u^2>$ as a function of temperature. One of the samples was bR-$NaClO_4$, measured in the 40–91 °C temperature range, the other one was bR-$NaCOOCH_3$ measured between 40 and 87 °C. Temperature scans were done upon increasing the temperature in steps of 7–10 °C below the transition region, and of 3–4 °C around the transition region. Additional vanadium, buffer and empty cell measurements were recorded for correction and normalization purposes [22].

In the examined time range (8 μeV energy resolution, corresponding to a time window of 100 ps through Heisenberg's uncertainty principle) on backscattering spectrometers, H-atom motions reflect the motions of the chemical groups to which they are bound [23]. The scattered elastic incoherent intensity can be described within the Gaussian approximation [24] by

$$I_{el}(Q, \varpi = 0 \pm \Delta E) \propto I_0 \exp\left(-\frac{1}{6}\left\langle u^2\right\rangle Q^2\right) \quad (1)$$

where I_0 is the intensity hitting the sample, $<u^2>$ is the average atomic mean square displacement, ω corresponds to the energy transfer, and Q to the momentum transfer between neutron and target. The average