Current Opinion in Colloid & Interface Science 20 (2015) 3–10

Contents lists available at ScienceDirect

Current Opinion in Colloid & Interface Science

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

Is the depletion force entropic?
Molecular crowding beyond steric interactions

Liel Sapir, Daniel Harries *

Institute of Chemistry and the Fritz Haber Research Center, The Hebrew University, Jerusalem, Israel

ARTICLE INFO

Article history:
Received 22 November 2014
Received in revised form 5 December 2014
Accepted 6 December 2014
Available online 15 December 2014

Keywords:
Depletion forces
Molecular crowding
Osmolytes
Protein folding
Cosolute effects
Preferential interaction
"Soft" interactions
Steric repulsions
Kirkwood-Buff solution theory

ABSTRACT

Cosolutes excluded from macromolecules create effective attractions between the excluding macromolecules, and promote their self-association. This "depletion force" serves an important stabilizing role in many biological and technological processes. Specifically, many osmolytes and polymeric crowders that are excluded from protein surfaces stabilize the more compact folded state. Yet, these excluded cosolutes operate through several, thermodynamically distinct, mechanisms. Here we review the experimentally found mechanisms and link these to possible underlying molecular interactions. One class of excluded cosolutes comprises protective osmolytes. These tend to induce attractive depletion forces that are enthalpically dominated but entropically destabilizing. Whereas polymeric crowders may act by similar mechanisms to osmolytes, in many other cases they induce depletion forces dominated by favorable entropy. These different thermodynamic mechanisms are intimately related to the effective interaction of cosolute with each macromolecule. The venerable Asakura-Oosawa model for depletion forces assumes that the cosolute-macromolecule interaction is entirely steric, thereby predicting fully entropic depletion forces. Augmenting this steric repulsion core with a "soft" repulsion shell adds an enthalpic contribution to the depletion force, which may even dominate all other contributions. Further, considering that cosolute-macromolecule effective interactions are temperature-dependent results in a depletion force that can be concertedly dominated by enthalpy and entropically disfavored, as observed experimentally for protective osmolytes. In this core-shell description, to account for depletion forces that are enthalpically dominated and entropically disfavored, it is sufficient to consider an effective microscopic cosolute-macromolecule soft shell that comprises an entropic attraction and an enthalpic repulsion. We show how the full gamut of cosolute effects can be rationalized using these simple considerations regarding the nature of the cosolute-macromolecule effective interaction.

1. Introduction

Emergence of life on earth probably owes much to the flexibility afforded by aqueous solutions to tweak macromolecular interactions. From changes in salinity to variations in pH, practically any solute (or "cosolute") that is added to solution alters the effective interactions between bathing macromolecules. Dating at least as far back as Hofmeister's experiments that involved the precipitation of proteins by the addition of salt to solution [1], for over a century the impact of cosolutes on macromolecular interactions is carefully being followed and documented. And in that time, variations in solution conditions have been repeatedly implemented as a strategy to modify macromolecular or colloidal interactions in numerous applications [2]. Gibbs lucidly determined that the excess or deficit of cosolute near an interface is in proportion to the change in free energy with cosolute concentration [3]. This relation is so instructively powerful that it has been developed by later researchers into a complete vocabulary that links cosolute preferential interactions with changes in macromolecular stability in solution [4–21].

Particularly interesting are cosolutes that, by virtue of their preferential exclusion from macromolecules, drive effective macromolecular attractions. The resulting "depletion force" dramatically impacts macromolecular stability and interactions. However, the link between free energy change and cosolute exclusion from macromolecular interfaces cannot unequivocally resolve the molecular mechanism for the observed cosolute effect. The first proposed molecular mechanism for the stabilizing effect of cosolutes is probably due to Asakura and Oosawa [22,23], who rationalized how cosolutes drive colloid coagulation through depletion, Fig. 1A. The attractively simple idea is that excluded cosolutes are sterically repelled from colloids or macromolecules. Therefore, there is a volume into which cosolutes cannot diffuse, thereby incurring a free energy cost in comparison with the same solution in the absence of macromolecules. If in any process the excluding interface is in some way minimized or "buried", part of the volume previously excluded from the cosolute's reach can be regained. This decrease in interfacial area can be achieved, for example, by macromolecular association or protein folding from its unfolded state, Fig. 1.

---

* Corresponding author.
E-mail address: daniel@fh.huji.ac.il (D. Harries).

http://dx.doi.org/10.1016/j.cocis.2014.12.003
1359-0294/

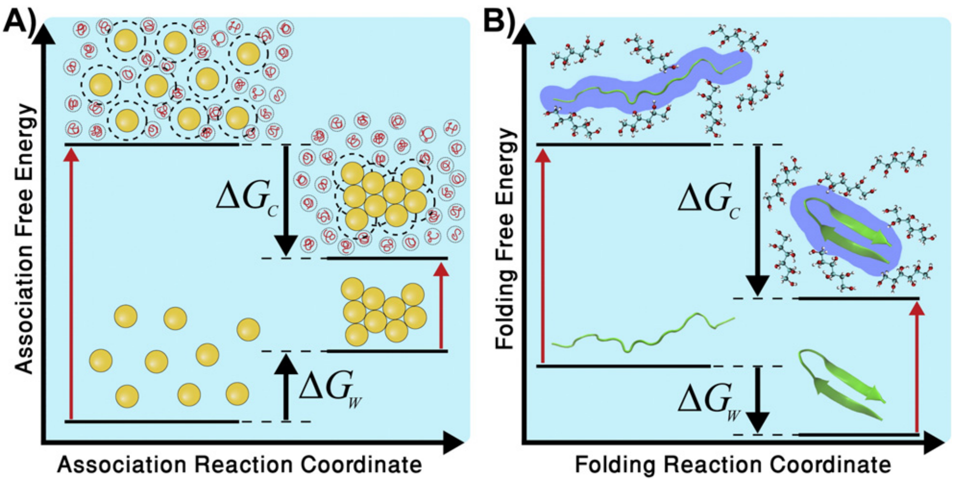

Fig. 1. Schematic free energy landscapes for two macromolecular processes, with and without excluded cosolutes. **(A)** Colloid coagulation in pure solvent (bottom) and in a polymeric "crowder" solution (top). **(B)** Protein folding in pure water or buffer (bottom) and in an osmolyte (sorbitol) solution (top). Each process incurs a free energy change ΔG_W in pure water, and a corresponding ΔG_C in the cosolute solution. For both processes (panels A,B) $\Delta G_C - \Delta G_W < 0$, since the excluded cosolutes destabilize the initial state more than the final state. The preferntial hydration volume is illustrated by the dashed line for the colloids in (A), and by the darker blue highlights for the protein in (B).

The resulting depletion force that Asakura and Oosawa predicted was completely entropic because it relied on fully steric interactions that are temperature independent. The net change in the stabilization free energy due to cosolute that accompanies the reduction in excluded volume is linearly dependent on that volume gain and on the osmotic pressure that pushes the macromolecules to associate. In the ideal van 't Hoff limit, this linearity also prescribes a linear change of free energy with temperature at a given cosolute concentration.

The Asakura-Oosawa model has been implemented on a large variety of processes, and often presents a good approximation, particularly when large polymeric crowders are involved in stabilizing colloids or proteins [2,24–28]. Over the years it was realized, however, that some cosolutes are not excluded purely because of steric interactions. Specifically, the free energy changes due to added cosolute were associated with a "sticky" or "soft" attraction component that mitigates the Asakura-Oosawa steric stabilization [29–39]. This additional attractive component is most probably the result of the complex interactions between cosolute, macromolecule, and water. The net emerging depletion force is entropically dominated (in the spirit of the Asakura-Oosawa model) but is counteracted to some extent by unfavorable enthalpy, due to cosolute attraction to the macromolecules.

Interestingly, this combined soft attraction shell and hard repulsion core is somewhat reminiscent of the mechanism by which chaotropic "sticky" ions show weaker salting out effects on macromolecules than the more strongly excluded "kosmotropes" [40]. This has led to occasional mention of "chaotropic" or "kosmotropic" molecularly neutral cosolutes. This nomenclature, borrowed from discussion of ions from the Hofmeister series to address neutral cosolutes, has some serious limitations because the electrostatic interactions that lead to ion exclusion are much different from steric interactions, and typically are strongly temperature dependent.

A more general and mechanistically satisfying description of cosolute action on macromolecules considers, beyond hard-cores, *attractive* or *repulsive* cosolute-macromolecule interactions (sometime referred to as "chemical interactions") [29,30,32,36,37,41]. These effective interactions are mediated by the solution components, but do not depend on the details of these interactions. Instead, they emerge as effective cosolute-macromolecule interactions. In contrast to soft attractions that tend to mitigate the depletion attraction between macromolecules, soft repulsive interactions were shown to enhance it [30,38,42,43].

Importantly, soft repulsions help rationalize the experimentally found impact of excluded cosolutes on protein stabilization. These experiments have revealed a complex reality, which at first glance fundamentally undermine the venerable Asakura-Oosawa model. Surprisingly, many excluded cosolutes, primarily osmolytes [44–46], display depletion forces that are entropically *disfavored*. The depletion force in these cases is enthalpically driven [47–50]. Important enthalpic and entropic contributions to excluded cosolutes' stabilization of proteins have also been found in molecular dynamics simulations that use atomistic force-fields with intermolecular interactions beyond hard-core [18,20,51,52].

We have recently shown that the discrepancy between the experimental excluded osmolyte effect on proteins and the steric Asakura-Oosawa model can be explained by considering an added soft repulsion shell to the hard-core repulsion between cosolute and macromolecule [53]. This addition can turn the depletion attraction into enthalpically dominated. Moreover, in the following sections we demonstrate how this enthalpically dominated depletion force can even become entropically *unfavorable* as long as the soft repulsion is temperature dependent.

We begin by presenting examples of experimental results highlighting the impact of excluded cosolutes on protein folding, and then turn to discuss the nature of the observed depletion forces. We survey prevalent entropically dominated mechanisms, and then review the emerging theories that allow to also explain enthalpically driven depletion forces. We demonstrate how enthalpic depletion forces naturally result from a simple consideration of effective soft repulsions between macromolecules and cosolute.

2. Cosolutes impact protein stability by several thermodynamically distinct mechanisms

Many cosolutes added to solution modulate macromolecular processes, such as colloid coagulation [22,23,54,55] or protein folding, Fig. 1 A,B. For example, cosolute-induced modifications to protein folding are witnessed as changes in the process free energy upon cosolute addition, $\Delta\Delta G = \Delta G_C - \Delta G_W$, where ΔG_W and ΔG_C are the folding free energies in pure water (or buffer) and in a solution containing an additional cosolute, respectively.

The effect of cosolute is often quantified using the preferential hydration coefficient, Γ_W, which scales as the variation of $\Delta\Delta G$ with osmolality, c_{Osm}. As established by the Gibbs adsorption isotherm [3], Γ_W describes the net excess or deficit in hydration around the macromolecule, which is necessarily related to the net exclusion or inclusion of cosolute molecules around that macromolecule. Applying this link