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Surface-driven denaturation of proteins during freeze-drying: An insight into the role of surfactants

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Abstract

Protein-based therapeutics may bind to interfaces during the freeze-drying process, possibly resulting in loss of activity. Here we investigate the mechanism by which surfactant molecules can counteract surface-induced denaturation through a detailed study of the stability of the GB1 peptide at the air-water, ice-water and silica-water interfaces. Using molecular dynamics simulations coupled with metadynamics we show that the amphiphilic nature of surfactants is key to their stabilizing/destabilizing effect, with an orientation-dependent mechanism in which the protein is stabilized when the hydrophilic heads of the surfactant point toward the protein.

Introduction

When proteins bind to interfaces, the resulting changes in protein structure can lead to loss of biological activity. For instance, the air-water, silica (glass)-water and ice-water surfaces are encountered during the freeze-drying process. In the present work, the folding behavior of a model peptide at these surfaces is investigated, in the presence and absence of the surfactant Tween 80. Using a molecular dynamics approach, we show that the extent to which surfactants prevent denaturation is dependent on the nature of the surface.[1]

Materials and Methods

Atomistic molecular dynamics (MD) simulations are coupled with the metadynamics enhanced sampling method [2] to investigate the effect of Tween 80 in preventing surface-induced denaturation of the GB1 peptide (shown in Fig. 1a). A variant of metadynamics called parallel bias metadynamics (PBMetaD) is used.[3]

Results and Discussion

We found that GB1 was destabilized at the air-water and ice-water interfaces, but stabilized at the silica surface. Tween 80 stabilized the protein at the air-water and ice-water surfaces (Fig. 1b), but slightly destabilized the protein at the silica interface. The surfactant molecules bound to the air and silica surface, while they clustered around the protein in the case of ice. An orientation-dependent mechanism of the surfactants was also identified, in which the protein was stabilized when the hydrophilic heads of the surfactant were oriented towards the protein, and destabilized when the hydrophobic tails pointed towards the peptide. The

latter orientation stabilized partially unfolded states of the protein, characterized by a larger non-polar surface area. The tails-toward-the-protein configuration is favored in a hydrophilic environment, explaining the mild destabilization observed at the silica-water interface. By contrast, the ice-water surface promotes the heads-toward-the-protein arrangement, that stabilizes the protein native structure. Finally, in the case of the air-water interface, the coating of the interface by the surfactant molecules, and the resulting inhibition of protein adsorption, accounts for the observed stabilization of the protein native structure.

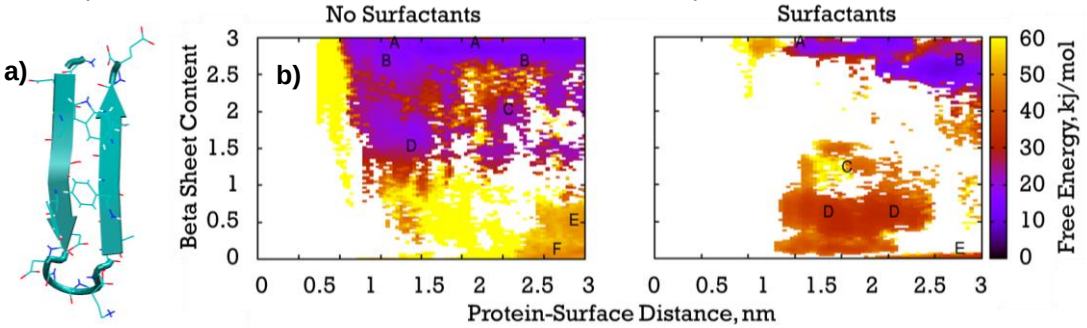

Fig. 1 *a) Cartoon structure of the GB1 hairpin. b) Free energy surface at the ice-water interface, in absence (left) or presence (right) of Tween 80.*

Conclusions

Our simulations suggest that the action of surfactants is complex; the amphiphilic nature of the surfactant, and its relative affinity for the protein and the surface, eventually determines the effect on the protein structure.

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