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Proteins in a brave new surfactant world☆

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ABSTRACT

This review discusses emerging topics within the field of protein–surfactant interactions over the last 4–5 years. The application of small-angle x-ray scattering has allowed us to construct ever more detailed models of the structures of different protein–surfactant complexes and has revealed common features shared between electrophoretic protein–SDS complexes and liprotides (complexes between lipids and partially denatured proteins), namely a generic core-shell structure which can also form beads on a string. SDS emerges as the best surfactant for gel electrophoresis from a series of studies comparing it with surfactants differing in chain length, degree of branching, and fluorination, as well as dodecyl sulfate with different counterions. Nevertheless, these surfactants possess useful properties for alternative applications. SDS also continues to serve as a useful tool for systematic folding/unfolding studies of membrane proteins together with the non-ionic surfactant dodecyl maltoside, as well as for studying hyperstable kinetically trapped proteins. Biosurfactants are coming to the fore as sustainable alternatives to chemical surfactants and show unique properties toward proteins that combine aspects of both ionic and non-ionic surfactants.

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1. Introduction

In 2017, we can celebrate the 50-year anniversary of the first report on the use of sodium dodecyl sulfate–polyacrylamide gel electrophoresis to separate proteins according to size [1]. Today SDS-PAGE is arguably the most widely used technique in protein science. Nevertheless, many aspects of the protein–surfactant interactions that underpin this technique remain unclear and controversial. This serves to illustrate that the effects of surfactants on protein conformation and stability are complex and varied. The study of these interactions remains an active field after decades of work and has in my view undergone a decided revival in the last decade or so, fuelled by a number of factors. Firstly, scientists have become adept at combining complementary techniques such as small-angle x-ray scattering (SAXS), isothermal titration calorimetry (ITC), and capillary electrophoresis (CE) to draw a much fuller picture of the individual steps involved in surfactant-induced protein unfolding. Small-angle scattering techniques in particular are making great methodological advances these years and are ideal for structural studies of protein–surfactant complexes whose size and dynamics defy atomic-resolution techniques such as NMR and crystallography. SAXS has also helped establish a link between protein–surfactant complexes and protein–oleic acid complexes that have attracted attention as potential cancer drugs. Secondly, the palette of surfactants has expanded greatly in scope and now includes branched surfactants, amphipols, and fluorinated surfactants. Another exciting newcomer to the field is the class of microbially produced biosurfactants whose biological origin, complex structure, and sustainable production make them very interesting. Finally, surfactants in mixed micelles remain so far the only way to carry out complete thermodynamic and kinetic analyses of the folding and unfolding of membrane proteins, a view that is confirmed by recent protein engineering studies of this very important class of proteins. This review will deal with all these aspects, focusing mainly on activities in the last 4 years and building on a review on protein–surfactant interactions that took us up to 2011 [2]. I also refer the interested reader to a 2010 review on the role of surfactants in protein aggregation [3]. I divide my topics into 4 areas, namely, SDS interactions with proteins, alternative surfactants (including biosurfactants), small-angle scattering studies of protein–surfactant complexes, and systematic use of surfactants for protein denaturation.

2. SDS interactions with proteins

2.1. How SDS binds to proteins: Teasing out the role of electrostatics and hydrophobics

We start with a study of fundamental interest: what makes monomeric (*i.e.* non-micellar) SDS bind to proteins? This is an important question since most proteins are denatured by SDS below its critical micelle concentration (cmc), making monomeric SDS the denaturing species. The answer may seem trivial: SDS has a negative head group that can interact with cationic side chains while its alkyl chain can contact hydrophobic regions. This is illustrated in the classic study by

☆ Soap and water and common sense are the best disinfectants.William Osler (1849–1919), co-founder of Johns Hopkins Medical School, clearly aware of the basic power of surfactants in many different contexts.

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Ada Yonath who soaked SDS into lysozyme crystals and localized individual SDS molecules bound via sulfate contacts to Arg or Lys side chains while the alkyl chains were buried in nearby hydrophobic regions [4]. However, a more subtle answer has been provided by the Whitesides group which has devoted considerable efforts to the role of electrostatics and hydrophobic interactions in protein–SDS interactions over the last decade, using a classical but convenient modification: acetylation of Lys side chain to remove electrostatic charges. Acetylation of amylase, for example, reduces SDS binding sufficiently to prevent its irreversible inactivation by SDS (though not cationic surfactants), but—rather surprisingly—also by non-ionic surfactants [5]. More detailed insight has been provided by the model protein ubiquitin, which has the attractive feature that it binds a large number of SDS molecules at sub-cmc concentrations without unfolding, allowing NMR studies to identify which regions of the protein come into contact with SDS [6]. It turns out that SDS binds to *hydrophobic* side chains in areas with positive surface potential, rather than simply binding to cationic side chains via long-range electrostatic effects. The authors explain this as efficient solvation of Lys/Arg side chains which reduces the electrostatic surface potential around these side chains sufficiently to shift binding to nearby hydrophobic surface chains. This is an important insight which illustrates the importance of global electrostatics and nicely corroborates an earlier comparative study on SDS-induced unfolding of two β-sheet proteins [7]; TII27 unfolds below the cmc and shows a more positive electrostatic potential than TNfn3 which only unfolds around the cmc. Conversely, a small change in pH from 8.0 to 6.0 can tip the potential sufficiently in a positive direction to increase SDS binding and promote unfolding of an otherwise SDS-resistant protein [8].

Binding to ubiquitin does not require any specific secondary structure (*e.g.* α-helices), though loop regions are disfavored, probably because a sufficiently stable docking platform has to form to accommodate SDS (there are no entropic advantages in the form of unfolding-induced dynamics, since the protein remains native at this stage). This study also illustrates that SDS-induced unfolding is not simple accumulation of SDS monomers on the ubiquitin surface. 11 SDS molecules bind to ubiquitin in the native state, and only the binding of 14 additional SDS molecules lead to unfolding [6]; if ubiquitin is acetylated, it does not bind SDS in the native state but unfolds directly upon binding 14 SDS molecules, effectively switching from three-state to two-state unfolding and showing that the denaturation step does not require prior "colonization" by monomeric SDS.

2.2. Studying kinetically stable proteins: Using SDS for conformational trapping

The insights into ubiquitin's multiple unfolding steps in the previous section were obtained using capillary electrophoresis (CE), which is a sensitive and versatile technique to follow the evolution of multiple species during SDS denaturation, as well as the stoichiometry of binding. CE has been used in a number of different protein–surfactant systems including the α-helical protein ACBP [9]. CE separates proteins according to their charge-to-mass ratio and not according to their mass since there is no gel matrix to sieve the proteins. This has been put to elegant use by Colón and co-workers to distinguish between native and denatured proteins [10]. All SDS-denatured proteins bind the same amount of SDS per mass (around 1.4 g SDS per g protein) and will therefore migrate together, while proteins which do not denature in SDS, and therefore bind less than 1.4 g/g, elute faster since they are not repelled so strongly by the cathode outlet. This distinction provides a very convenient tool to study kinetically stable proteins, *i.e.* hyperstable proteins which remain trapped in the native state due to an unusually high kinetic barrier to unfolding. Such proteins can have unfolding half-lives of many years, which is biologically significant since it allows them to resist proteolysis and formation of aggregation-prone states. SDS has proven very useful to detect and analyze these proteins, because they typically only unfold in SDS when boiled.

The method can even be used on complex biological samples using 2D SDS-PAGE [11]. In the first dimension, proteins are separated by conventional SDS-PAGE but—importantly—without boiling them beforehand. The strip is then boiled in SDS for 10 minutes before being placed above a larger gel for a second-dimension run. Proteins that already denatured in the first run will not denature further. Their migration will not change and they will therefore form a diagonal. In contrast, kinetically stable proteins only unfold in the boiling step and will therefore migrate to an off-diagonal position (usually below the diagonal), allowing subsequent identification by MS. Potential false positives, such as highly negatively charged proteins which bind less SDS, or components in kinetically stable multi-component complexes, can be filtered out in subsequent validation steps. Intuitively one might expect SDS to unfold these proteins at high temperatures because it destabilizes the proteins sufficiently to reduce the melting temperature below 100 °C. This may not be the case; rather, SDS may simply trap the protein in the denatured state by binding to hydrophobic surfaces exposed by unfolding, without directly interfering with the kinetics of unfolding. In this way, SDS can reduce melting temperatures without affecting unfolding kinetics simply because it makes unfolding irreversible.

SDS-PAGE has long been used to monitor refolding of β-barrel structured outer membrane proteins such as OmpA, since the native state of OmpA resists unfolding in SDS while the denatured state is prevented from folding to the native state [12]. This is also illustrated very well in a more recent study by the Colón group [13]. Hyperstable proteins such as superoxide dismutase or transthyretin are incubated with SDS at elevated temperatures such as 70 °C, and samples can then be removed and monitored by SDS-PAGE which can also quantify the amount of folded and unfolded protein. Another advantage of this approach is that it can deal with relatively impure protein samples as long as the bands corresponding to folded and unfolded protein can be determined unquivocally. Importantly, the authors show that the unfolding rate constants k_{unfold} obtained by SDS-PAGE are identical to those obtained by direct circular dichroism measurements, confirming the trapping hypothesis. This makes the process analogous to an hydrogen–deuterium exchange mechanism $\mathrm{N} \underset{k_f}{\overset{k_u}{\rightleftarrows}} \mathrm{U} \overset{k_t}{\rightarrow} \mathrm{C}$ in the so-called EX1 limit where N is the native state, U the unfolded state and C is the exchanged (or otherwise irreversibly trapped) state. In this scheme the observed rate constant $k_{t,obs} = k_u k_t/(k_t + k_f) \approx k_u$ since $k_t >> k_f$. Unfolding kinetics at 37 °C can be estimated by measuring the kinetics at different temperatures and then extrapolating to lower temperatures in Eyring plots ($\ln(k_u/T)$ versus $1/T$).

2.3. The importance of being accompanied: The role of counterions in unfolding

When it comes to varying surfactant properties to modulate protein–surfactant interactions, the traditional approach has been to vary alkyl chain lengths or head group compositions (see Section 2.4). Much less attention has been brought to the role of counterions, but this has been rectified by another recent study by the Whitesides group [14]. While dodecyl sulfate anions (DS^-) in the monomeric state are strong electrolytes which are virtually 100% dissociated from their counterions, the micellar state is only partially dissociated: electrical conductivity measurements show that DS^- is 77% neutralized by Na^+ [15]. If Na^+ is replaced by NR_4^+ ions (where R is C_1–C_4), there is even closer association of counterions, probably due to hydrophobic interactions, though the same types of micelles are formed. The interesting twist is that the closer NR_4^+ association lowers the cmc and thus restricts the monomeric concentration range. Bovine carbonic anhydrase (BCA) is denatured by monomeric and not micellar DS^-, showing a linear relationship between $\log(k_u)$ and $[DS^-]$ right up to the cmc—a denaturant-like phenomenon also seen for other proteins such as

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