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Proteins and biosurfactants: Structures, functions, and recent applications

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Abstract

Synergies between surfactants and proteins are found everywhere in everyday life. Beneficial interactions are exploited in fields such as food processing, pharmaceutical production, and laundry, leading to better products and lower energy consumption. Nevertheless, there is still room for improvement regarding sustainability. Here, biosurfactants (BS) are an attractive alternative to petrochemical surfactants. Insights into BS-protein interactions can help replacing traditional surfactants with BS and uncover new opportunities. Here, we review recent work on proteins' interactions with BS, with focus on the self-assembly of protein:BS complexes and BS' effects on enzymatic activity. Generally, interactions are milder than those with traditional ionic surfactants, leading to modest effects on protein structure and self-assembly, while enzymatic inhibition is generally observed above BS' critical micelle concentration. Mild interactions between proteins and BS show promise in forming functional complexes with proteins, however, further studies are required to understand and minimize the detrimental effects that do occur.

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Biosurfactants, Protein, Enzyme, Interaction, Surfactants.

Abbreviations

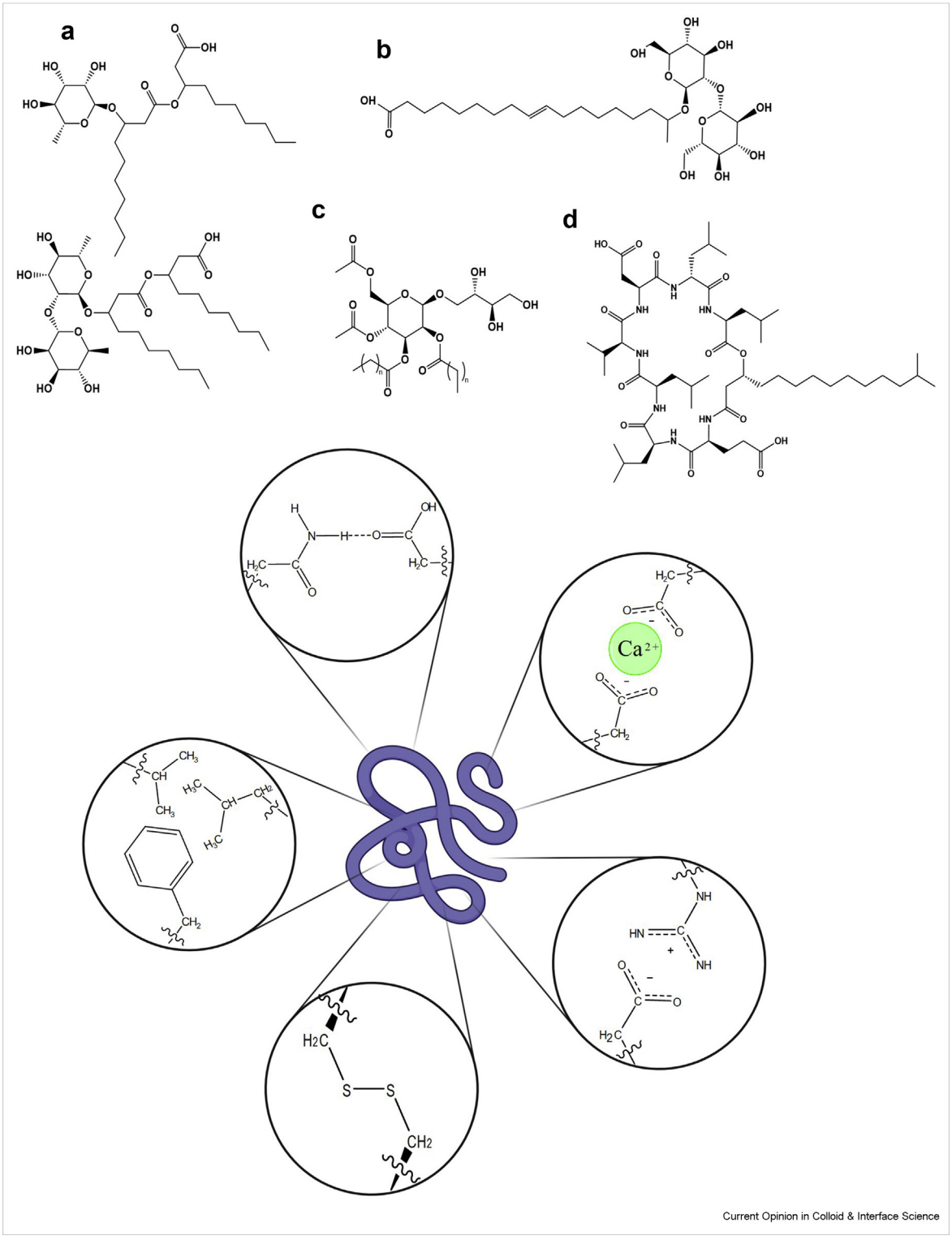
BS, biosurfactants; RL, rhamnolipid; SL, sophorolipid; MEL-A, mannosylerythritol lipids-A; SDS, sodium dodecyl sulfate; CMC, critical micelle concentration; SANS/SAXS, small-angle neutrons/X-ray scattering; DLS, dynamic light scattering; ITC, isothermal titration calorimetry; TEM, transmission electron microscopy; SEM, scanning electron microscopy; CLSM, confocal laser scanning microscopy.

Introduction

Surface active agents (surfactants) are molecules with a preference for surfaces and interfaces thanks to their amphiphilic composition, consisting of two different moieties, a hydrophilic (charged or polar) head, and a hydrophobic tail. They self-assemble into higher-order structures (micelles or vesicles) above a certain concentration (critical micelle concentration, CMC), driven by the hydrophobicity of their aliphatic chain and balanced by the size and properties of their head group. Surfactants have found a great variety of applications and uses in the everyday life of consumers, exploiting their amphiphilic properties. Most current surfactants are synthesized from petrochemical sources. In contrast, biosurfactants (BS) are produced by various microorganisms through different metabolic pathways involving secondary metabolites, and constitute a promising green alternative to petrochemical surfactants (here referred to as p-surfactants; the term "surfactant" encompasses both BS and p-surfactants). BS' polar heads are derived from biomolecules such as saccharides or peptides, improving biodegradability compared to p-surfactants and leading to greater chemical variability. Examples of these somewhat unusual molecules include branched structures (rhamnolipids (RL)), bola amphiphiles (sophorolipids (SL)), or surfactants with flexible polar heads (surfactin), which translate into different self-assemblies that can change in response to the environment (Figure 1). BS self-assemblies have previously been reviewed in detail [1]. The different chemical properties of these surfactants not only promote differences in the self-assembled structures but can also translate into different interactions and complexes of BS with proteins.

Most surfactants interact with proteins as part of their applications in *e.g.* food processing, pharmaceutical

Figure 1



Top - Structures of most reported (and mentioned in the review) biosurfactants. (**a**) Mono and di-rhamnolipids. (**b**) Sophorolipid (C18-1). (**c**) Mannosylerythritol Lipids-A. (**d**) Surfactin. Bottom - Depiction of major protein interactions responsible for folding and self-assembly.

formulations and detergency. These interactions, and thus their applications, are greatly influenced by their amphiphilic properties. A basic understanding of the interactions between surfactants and proteins is therefore necessary to better understand and expand the current range of applications. The most common techniques used for studying the interactions between proteins and BS include:

1) **Spectroscopy** (fluorescence, circular dichroism (CD), and more rarely infrared[†]) monitors changes in the secondary and tertiary structure of proteins as well as binding modes and conformational changes of proteins upon interaction with surfactants.
2) **Microcalorimetry** (isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC)) generally measure the thermodynamic properties of protein-ligand interactions and the stability of the complexes formed through protein-surfactant interactions, leading to binding stoichiometries, binding constants and energies (including enthalpy and entropy) of formation of protein-surfactant complexes.
3) Amongst **computational approaches**, molecular docking can predict binding modes and binding energies of protein-ligand complexes, while molecular dynamics simulations (nowadays always performed at full atomistic resolution) provide information on the dynamic behavior and stability of protein-ligand complexes over time.
4) **Scattering techniques** (dynamic and static light scattering (DLS and SLS) and small-angle X-ray/neutron scattering (SAXS/SANS)) provide low-resolution information such as the size, shape, and internal organization and co-existence of complexes and how they change in solution.
5) **Microscopy** (transmission electron microscopy (TEM), scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM), and cryo-TEM) gives direct information about the different structures that are formed as a result of the protein:BS interactions. Cryo-TEM can potentially provide atomic-level resolution (provided stable structures are available) with barely any sample preparation, having little or no effect on the resulting information (in contrast to techniques where crystallization, labeling, coating, or drying is required and might interfere with the resulting information). However, this requires homogeneous and relatively static structures [2], a feature rarely seen for protein-surfactant complexes.
6) Finally, **separation-based techniques** such as capillary zone electrophoresis can provide complementary information on the co-existence and sizes of different protein-surfactant complexes formed at different protein-surfactant stoichiometries [3].

Ideally, experimental and computational techniques should be combined to provide a fuller picture of protein-surfactant interactions, the underlying mechanisms, and their potential applications. We have very recently described these techniques and their deployment in the development of a general model for the denaturation of proteins by the widely used anionic surfactant SDS (sodium dodecyl sulfate) [4], building on an even earlier very comprehensive review [5], while more specific aspects of biosurfactants have been described in Refs. [6,7].

Here we outline the major forces driving BS protein interactions and their impact on the stability and functionality of protein:BS complexes as well as protein conformational changes and enzymatic activation/inhibition. We conclude with a discussion of recent studies involving BS and proteins (as well as other additives) in terms of possible applications and future prospects.

Major interactions between biosurfactants and proteins

Electrostatic interactions, hydrogen bonding, hydrophobic interactions, and van der Waals forces all contribute to the stability and functionality of protein-molecule complexes. In some cases, the type and strength of the driving forces can also influence the conformational changes of proteins and their subsequent (lack of) interactions with other molecules. BS self-assembly has been proven to be similar to, yet distinct from, that of p-surfactants. Supramolecular (*e.g.*, micellar) formation is driven by hydrophobic interactions but modulated by the polar head(s), which is more complex for BS (Figure 1) and gives rise to more types of interactions with proteins. The metabolite-based polar head includes a greater amount of hydrogen bond donor and acceptor groups and an acidic group (carboxylate) with a pK_a in the weakly acidic range with chelating affinity. This is relevant for applications in health, pharmacy, and laundry sectors in terms of pH range of applications. The carboxylate group, which predominates under alkaline conditions, has a negative charge distributed between the two oxygen atoms, and this leads to a more delocalized negative charge in the polar head than *e.g.*, the "hard" sulfate group of a p-surfactant. In turn this reduces the ratio between charge and BS polar head volume, which is responsible for the low CMC values observed for BS, since charge repulsion between the polar heads is greatly decreased compared with classical ionic p-surfactants. Consequently, the value of the CMC is generally one or several orders of magnitude lower for BS compared with their p-surfactant counterparts (depending on physicochemical parameters of the solution such as ionic strength, pH or presence of divalent cations).

[†] The most common type of IR, such as Attenuated Total Reflection Fourier Transform IR (ATR-FTIR) involves drying the sample to remove the water signal. As well as affecting protein conformation, this drying process makes it meaningless to talk about surfactant concentrations and the impact of micelle formation since there is no aqueous medium to generate micelles.

This broad toolbox of parameters may be tuned and optimized to achieve desired morphologies in the BS self-assembly: An extensive overview of reported structures, hydrophilic-lipophilic balances (HLB) and CMC values is provided in a recent comprehensive review [1] and the impact of the above mentioned physicochemical parameters on BS structures have recently been described [8]. This complex behavior can also be expected to be reflected in the interactions of the BS with globular proteins and other structures like fibers or glycans in glycosylated proteins. Protein self-assembly, *e.g.*, folding, oligomerization and fibrillation, occurs in a similar manner. Both surfactant−surfactant and protein−protein interactions are driven by a complex combination of different non-covalent interactions such as the hydrophobic effect, coulombic interactions, hydrogen bonding, metal chelation, van der Waals forces and other weak but abundant secondary forces [9]. In addition, proteins can be modulated by covalent bonding such as disulfide bridges and post-translational modifications including phosphorylation and glycosylation, which can also affect their interactions with surfactants, although there are only few studies on this [10].

Generally, protein interactions with BS are much weaker than those involving p-surfactants like SDS, just as is seen for ethoxylated alcohols and other non-ionic surfactants. Although many BS are charged, electrostatic contributions are weakened due to charge delocalization. BS-protein studies generally involve fluorescence spectroscopy since the interaction is so weak - and the CMC values so low - that ITC does not provide reliable stoichiometric binding data, in contrast to the higher (and more easily measured) values for p-surfactants [11]. Since the resulting heat is directly proportional to the concentration of each species (injectant/ligand and macromolecule), injection of BS to achieve a concentration below CMC does not result in significant heat values; consequently, the heat related to the (weak) interaction is not easily distinguishable from the demicellization or the micelle dilution heat.

Briefly put, BS-protein interactions may have a direct effect on the protein structure, but it is generally not strong enough to cause denaturation or other thermodynamically visible effects measurable by calorimetry. It should also be noted that interfacial effects related to the amphiphilic behavior of BS, such as adsorption or desorption, have a great impact in protein functions as will be described in the next sections.

Self-assembly of biosurfactants and proteins

Interactions between any surfactant and a protein through the forces mentioned in the previous sections may have different effects on the resulting structure. Depending on the ratio between protein and surfactant, the protein can have conformational changes that range from stabilization to even denaturation upon binding of surfactant molecules. Conversely, surfactant self-assembly can be tuned by proteins in ensembles such as protein-decorated micelles. Higher-order complexes may also be formed (or tuned) by surfactant-protein interactions, which includes hierarchical structures such as amyloid fibrils and other possible biomaterials such as hydrogels or nanocarriers relevant for different applications.

Small changes in protein structure and function compared to SDS

The binding of surfactant molecules to proteins may have an implication on the protein structure (secondary, tertiary, quaternary or ultrastructure) and its consequent function. Several molecular simulations (all at atomistic resolution) have investigated how binding of a BS lipopeptide molecule can affect different protein functions. These include binding to:

- The tubulin binding site [12], required for the controlled oligomerization and further fibrillation.
- The active site of different heat shock protein 90 (Hsp90) [13], inhibiting ATPase activity and consequent microbial growth.
- The hydrophobic pockets of soy protein isolate [14], affecting protein flexibility and stability.

These simulation studies, however, generally lack further experimental information about how this binding may promote other possible cooperative conformational changes, nor do they address the effect of micelle formation of the BS and possible protein:BS complex formation.

Experimentally, different BS have shown a weak effect on glucagon solubilization, displaying only a small helicity induction compared to the observed for SDS [11]. This effect on the secondary structure is also observed for casein micelles: While SDS induces α-helix formation [15], BS have no clear effect on their secondary structure. Similarly, when mannosylerythritol lipids-A (MEL-A) is used for controlling β-lactoglobulin (β-lg) heat-induced aggregation [16], it decreases the helicity percentage. However, MEL-A stabilizes the helix content during heat treatments, resulting in a controlled microscale core-shell vesicle formation of heat-induced β-lg:MEL-A complexes instead of the larger aggregates formed by β-lg on its own. This controlled self-assembly improves several interfacial properties such as flexible structures, interface activities, and foaming or emulsifying capacities.

BS can also compete with protein cofactors such as divalent ions, normally coordinated by a deprotonated carboxylate group. A relevant example is provided by a

study of the interactions between bovine serum albumin and surfactin in the presence of different divalent cations [17]. The study demonstrates experimentally and using computer simulations that the presence of different divalent ions (Cu^{2+}, Zn^{2+}, Mg^{2+} and Ca^{2+}) promotes the reduction of surface tension while diminishing the binding constant and changing the geometrical orientations of the surfactin binding with the subdomain IIA of BSA. Remarkably, electrostatics (<1% of the total binding free energy) contribute only weakly to binding, while van der Waals contacts, hydrogen bonding and desolvation (hydrophobic interactions) are the major forces driving binding. Further characterization of the self-assembled structure by means of in-solution techniques like cryo-EM or SAXS/SANS would be of great interest to confirm docking studies and elucidate the influence of these competing interactions in the final structure.

Changes in hierarchical structures

Higher-order ensembles based on protein composition show great potential as biomaterials like tissues or membranes for applications within *e.g.* biomedicine and water treatment. These materials mimic naturally occurring biofilms, where amyloid fibrils and other exopolymeric substances are major components. BS can affect the protein self-assembly through interactions with the enzymes or the surrounding environment. Surface coating by BS, especially surfactin, inhibits biofilm adhesion [18]. However, the interaction of the BS with different exopolymeric substances may affect the protein hierarchical structure and properties in different manners. For examples, protein fibrils formed by bacterial amyloids can be targeted by BS. Modulation of amyloid formation by bacterial proteins such as FapC by RL [19] can affect biofilm formation and thus potentially bacterial infections. RL did not affect fibril morphology and structure but has a stimulating effect on aggregation kinetics, reducing the lag phase in the formation of the fibril. Though mediated by weak interaction according to ITC, these interactions are similar to the ones observed for SDS, promoting β-sheet structures that are characteristic of the amyloid fibril. However, an excess of SDS promotes α-helix formation while the major secondary structure for RL-induced fibrils is not shifted. Interestingly, at high concentrations of the BS, the model used for describing the scattering profile is that of a protein-decorated cylindrical micelle. This is similar to other complexes described previously for p-surfactants [20] but reflects the different self-assembly of this BS while retaining the protein ability for fibrillation, as observed through TEM and fibrillation assays.

Another natural amyloidogenic protein is silk fibroin, a two-chain protein of approximately 416 kDa that forms fishnet-like fibril structures able to gel to hydrogels through refolding of the disordered secondary structure into intermolecular β-sheets [21]. SL accelerates the hydrogel formation kinetics [22] from 20 to 8 days at the maximum SL concentration studied. As expected, this acceleration occurs through weak interactions between the BS and the protein in which the BS are depleting the water molecules present through hydrophobic interactions, acting as a scaffold. However, the structures formed by the surfactants do not disassemble but coexist with the protein hydrogel. Dubey et al. [23] have reported a similar insight on the system for which an alkaline pH is a determining factor for the gelation process, promoting SL deprotonation, therefore suggesting an electrostatic requirement for the self-assembly acceleration.

These studies indicate that BS does not inhibit biofilm by blocking the exopolymeric fibrillar self-assembly. Rather, fibrillation kinetics are increased, apparently due to the desolvation effect in the vicinity of the fibrils that act as a scaffold, promoting self-assembly through weak interactions. Biofilm inhibition may be attributed to the decrease in the surface hydrophobicity from BS coating, therefore blocking microbial adhesion and growth.

Effects of biosurfactants on enzymatic kinetics

Enzymes, which are Nature's most efficient catalysts, are fundamental to different industrial applications. They help decrease energy consumption and overall pollution for societally important processes within chemical and food industries, and even plastic degradation [24]. Somewhat confusingly, BS can both inhibit and activate enzymes.

The Sabatier principle defines optimal activity as a "sweet spot" where the enzyme binds tightly enough to the substrate to be able to catalyze a reaction but not so tightly that it is difficult for it to be released afterwards [25]. Surfactants (and BS specifically) can influence (positively or negatively) the protein affinity towards the substrate (access, binding or release) in different manners (Figure 2):

- Conformational changes (either active site or the surroundings) in the protein structure promoted by BS interaction may lead to activity related changes such as a change in the protein flexibility (hydrophobic stabilization or hydrogen bonding partial disruption), metal sequestering or even denaturation.
- Interaction of the BS with the substrate or the media can promote changes in enzymatic activity affecting enzyme-substrate complex formation or release. First, the increase in solubility or promotion of substrate dispersion due to the presence of surfactants will intrinsically increase enzymatic activity. Secondly, decrease of the surface tension or alterations in the

Figure 2

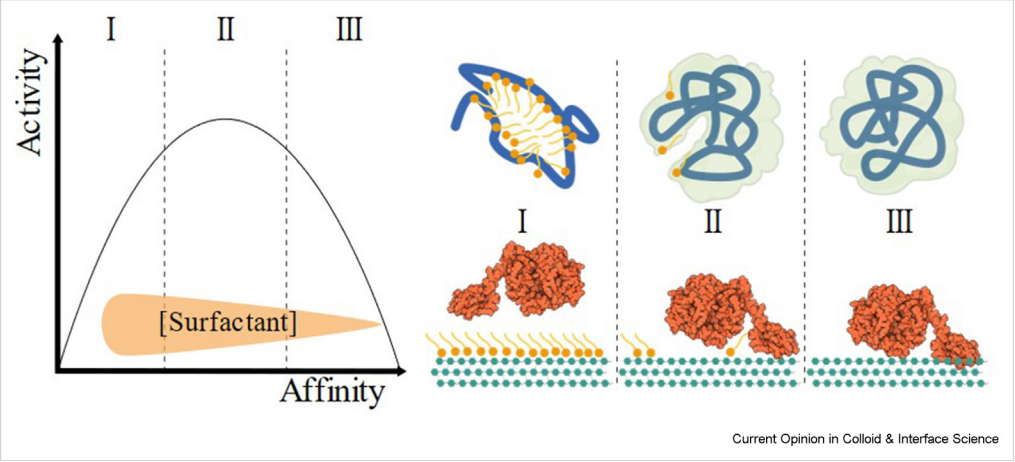


Illustration of how the presence of surfactants (both BS and p-surfactants) and related interfacial effects may influence substrate affinity and therefore enzymatic activity. (I) Low affinity between an enzyme and the substrate can be achieved at high surfactant concentration, either through protein denaturation (top) or substrate inaccessibility (bottom). (II) Optimal enzymatic activity can be facilitated by minor conformational changes in the protein that allow improved entrance to the active site and later release (top) or reduction of the substrate binding strength, and thus increased ease of dissociation, due to limited steric hindrance by the substrate (bottom). (III) In the absence of surfactants, a decrease in the activity could be caused by a native enzyme with an inaccessible active site (top) or strong binding to the surface (bottom).

electrostatic interactions, ionic strength, or other physical parameters may also affect catalytic activity.

Enzymatic inhibition by BS

BS can broadly speaking inhibit enzymatic activity by blocking the active site (or access to it) or by protein stabilization, leading to a less flexible structure. Generally, BS inhibit enzymes. Some clear examples are β-glucosidase [26], ATPase [27], or Hsp90 [13]. Interestingly, all these inhibitions occur at a BS concentration close to or above the reported CMC value. Therefore, docking simulation studies involving only one or a few BS molecules are inappropriate. Considering the micellar structures formed at the protein:BS ratios at which the enzymes are being inhibited, BS should be simulated as micelles or hemimicelles and not as monomers.

β-glucosidase, an enzyme with a broad range of applications in food and biotechnology industries, interacts detrimentally with MEL-A [26], losing enzymatic activity despite an increase in the thermal stability of the protein. Interestingly, the shift in secondary structure towards α-helix observed for an intermediate BS concentration increases the protein activity while higher BS concentrations recover the initial secondary structure of the native protein with partial inhibition of the enzymatic activity. The suggested mechanism of inhibition is the binding between the enzyme active site and the BS, although the concentrations and ratios of the inhibition indicate the presence of micelles and not surfactant monomers.

Partial inhibition is similarly described for an alkaline commercial protease from *Bacillus subtilis* [28] and a laccase from *T. versicolor* [29] in the presence of surfactin and RL, respectively. The proteolytic activity of a protease decreases around the CMC after an initial proteolytic activation (∼20%) at low surfactin concentrations. Similarly, the laccase shows maximum degradation of bisphenol A at a concentration below the CMC, after which further addition of RL reduces the kinetics of degradation. In the first study, the proposed model for the interaction is a partial denaturation, however, the model does not agree with the lack of change in the protein secondary structure. In our view, the reasoning given by Onaizi and Alshabib [29] is more adequate. In this model, the negative ion accumulation resulting from the micellization, probably in the vicinity of the active site of the laccase, sterically reduces accessibility and therefore causes partial inhibition.

Total inhibition by surfactin is observed for Hsp90 from the cyanobacterium *S. Elongatus* [13]. Other lipopeptides show partial inhibition but only surfactin completely inhibits the ATPase activity of the enzyme, suggesting an irreversible binding that agrees with the computational results. Hsp90 from *E. coli* or *S. pombe* are not inhibited, which indicates a highly specific interaction of surfactin with the ATP binding site of this protein.

Further study of these systems can potentially explain why the enzymatic activities are not completely lost if a binding is occurring as often suggested. The

concentration of surfactant at which enzymatic inhibition occurs is generally above both the enzyme concentration and CMC which may suggest a dynamic equilibrium between bonded and micellar BS, nonetheless, it still fails to explain the reported activation and changes in the secondary structure below the CMC, indicating different interactions for monomers and micelles. Additionally, proteins may be susceptible to the presence of different divalent ions [30] and other cofactors, which also may interact with certain functional groups in the BS. This competition may be an indication of an inhibiting interaction.

Activation through interfacial effects

The ability of surfactants to position at the interface and reduce surface tension can promote enzymatic activity in different ways. First, activation may occur through conformational changes caused by the interaction of the enzyme with the surfactant such as lipase lid opening [20] or an increase in protein flexibility promoted by a weak destabilization. Secondly, the reduction of surface tension can increase accessibility (therefore promoting adsorption) or decrease the affinity of the enzymes for insoluble substrates (such as cellulose or plastics), promoting desorption. Both effects can translate into an increase in activity.

As regards activation of enzymes by conformational changes, a lipase from *Yarrowia lipolytica* shows high tolerance to two different lipopeptides, amphisin, and viscosinamide [31]. The enzyme is activated in the presence of both BS through the apparent formation of micellar aggregates. These results indicate a great potential for future complex applications in fields like detergents, pharmaceutical, or food industries. Moreover, it shows the potential of BS-functional enzymes, arising by microbial growth in the presence of BS [32], in the presence of harsh environments that may promote BS-growing microorganisms [33], or through screening of enzymes from known BS-producing microorganisms [34,35].

Interfacial activation from BS has potential implications in fields such as food and biotechnology industries or environmental remediation. Enzymatic degradation of lignocellulosic biomass (a complex material composed of different ratios of cellulose, lignin, and hemicellulose) improves with interfacial activation [36] by the presence of BS. The addition of SL reduced the non-specific adsorption of cellulase, cellobiase, and hemicellulase, with a major effect on lignin adsorption. Moreover, the weak interactions between BS and the enzymes translate into a lack of denaturation of the enzymes responsible for the degradation, contrary to what may occur with other p-surfactants.

Interfacial activation of lignocellulose by BS illustrates how interfacial phenomena can promote enzymatic activities. Thus, degradation of other complex materials such as plastics [37], petroleum [38], or coatings may be improved in the future thanks to the synergies between enzymes and BS. A specific review of the topic of surfactant-enhanced enzymatic hydrolysis of biomass has been published recently [39].

Applications of biosurfactant-protein complexes

The previous sections highlighted the general effects in catalysis and self-assembly that some protein:BS displays. This section reviews and discusses the actual applications recently proposed for some of these complexes, either for protein:BS systems by themselves or formulated in synergy with other components. First, in emulsions, BS improves the miscibility of two otherwise immiscible surfaces, improving the emulsion capacities of protein-only systems, while for nanocarriers, BS acts as flexible patches between the protein (and other components), improving the properties of the self-assembled structure.

For both applications, researchers take advantage of the adsorption-prone behavior of the BS, together with its weak interactions with protein to obtain greater structural flexibility and stability than the protein-based surface alone, leading to complexes useful as emulsion stabilizers [40] and nanocarriers [41]. The resulting structures are similar to what would be expected for non-ionic surfactants (in terms of concentration) but display low tendency to flocculate or aggregate thanks to the delocalization of the charge in the polar head and the consequent weak electrostatic repulsion.

Emulsifiers and foaming agents

In the food and cosmetic industries, BS and protein:BS mixtures are commonly used as emulsifiers or foaming agents. The surfactant behavior of the system together with the antimicrobial properties of BS makes them a promising agent for this application. RL shifts the secondary structure of the soy protein towards β-sheet [40]. The complex shows an improvement in foam capacity and stabilization due to the protein change in the secondary structure, lowering structural rigidity and achieving an interfacial activation. Lactonic SL acts differently on a soy protein isolate [14], promoting α-helix secondary structure with an increasing concentration of the BS. The result is also an increase in foam capacity and stabilization, similar to RL. However, contrary to what is commonly reported, no significant antibacterial effects are observed. RL have a similar effect in complex with egg white protein [42]; here the effect on the secondary structure has not been reported, however, the amount of oil incorporated in the emulsion increases with RL concentration once a certain value (probably corresponding to the CMC) is reached. From a macroscopic perspective, the resulting droplets show a

lower particle size while achieving a thin and soft interface film, reaching a gel-like network when increasing RL concentration.

Nanocarriers

A final mention of potential pharmaceutical research applications is for the BS to be used as model systems or complementing components towards membrane models. This can help further studies of membrane proteins or the use of nanocarriers with promising cargo delivery capacities [43,44], driven by their ability to fuse with membranes and induce pore formation [45,46]. Their interaction with membranes is beyond the scope of the current text but shows potential in the design of different nanocarriers. The previously described interactions found within BS (hydrogen bonding, hydrophobic effect, and electrostatic interactions) are responsible for improving the desired nanocarrier properties.

Synergies between protein elements and BS, often paired with polysaccharides in ternary systems, improves the cargo loading capacities and properties of the different carriers. Ternary nanocarriers containing the maize protein zein, propylene glycol alginate, and RL [47] have desirable properties regarding physical parameters like pH and ionic strength. The carriers improve encapsulation and bioaccessibility while protecting cargoes such as nutritionally important antioxidant phenolics like curcumin [48] resveratrol [49] or coenzyme Q10 [41,50]. These compounds have also been incorporated into carriers made with pea protein isolate and the polysaccharide high methoxyl pectin using a different fabrication method that only requires changes in the pH and not ethanol [51–53]. This method efficiently promotes encapsulation of the cargoes when combined with different surfactants like RL, tea saponin, and ethyl lauroyl arginate hydrochloride. Among these, RL is best both for encapsulation capacity and stability (thermal resistance and cargo protection).

While RLs are the most highly studied BS, similar composite nanoparticles of zein and chondroitin sulfate show improved encapsulation of curcumin in the presence of SL [54]. Toxicity studies will be required, though, since they have been proven to have anticancer activity. Furthermore, a similar nano-complex shows growth inhibition against *Candida albicans* [55] by biofilm and hyphal repression, and other lipopeptides' effect on different plant diseases has been reviewed recently [56].

Concluding remarks

The proven and promising application of the complexes between BS and proteins requires us to improve our understanding of their interactions and how they are affected by other components in complex formulations. Interactions and competition with Ca^{2+} and other ions or small molecules influence the BS:protein complexes, and it is of crucial importance in fields such as cosmetics or the laundry industry. Fields in which p-surfactants have proven useful will require further study of the complex formulations containing BS to ensure the feasibility of the replacement. Understanding the complexes formed and how to tune them using physicochemical parameters or other additives opens a myriad of possibilities for application design. Furthermore, compelling outcomes may result from a partial replacement that can promote synergies between both BS and p-surfactants.

Tuning enzymatic affinity towards different substrates using BS and p-surfactants will be useful during the enzymatic cleavage of persistent and harmful substrates such as plastics or lignocellulosic materials. BS are effective bioremediation agents, and the synergies that arise with proteins can lead to higher efficiency for different contaminants. Thus the surfactant-like behavior of per- and polyfluoroalkyls substances (PFAS) [57] could be exploited by the usage of BS to sequester (and concentrate) them before their degradation, for which enzymatic cleavage is being investigated [58].

Further applications of the BS arise from these natural amphiphiles displaying promising applications as antibiotics, inhibiting biofilm and exopolymeric substances growth, anticarcinogenic activity, or acting as crop pesticides. Understanding the mechanism behind these biologically relevant applications, considering that interactions with relevant proteins could be crucial and potentially improved using them as double agents as nanocarriers and therapeutic factor, is an interesting outcome worth pursuing further by investigating their toxicity and mechanism of action.

Finally, the ability of BS to decrease the time required for the self-assembly of protein-based hydrogels highlights BS as a promising application for related materials. Further studies of the rheological and possible catalytical properties of these hydrogels or similar membranes would be of great interest to determine how the presence of BS can affect their formation and ensuing properties.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Figures were created using BioRender (biorender.com).

Data availability

No data was used for the research described in the article.

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