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Solubilization of Elemental Sulfur by Surfactants Promotes Reduction to H_2S by Thiols

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Elemental sulfur (S_8) may contribute to sulfane sulfur (S^0) storage in biological systems. We demonstrate that surfactants can solubilize S_8 in water and promote S_8 reduction to H_2S by thiols. Moreover, anionic and cationic surfactants interact differently with intermediate S^0 carriers, highlighting how specific hydrophobic microenvironments impact reactive sulfur species.

Reactive sulfur species (RSS), such as hydrogen sulfide (H_2S), persulfides (RSSH), and hydropolysulfides ($RS(S)_nSH$) play pivotal roles in redox biology. For example, H_2S is the most recently recognized gasotransmitter, contains a sulfur atom in the most reduced S^{2-} state, and has been studied extensively as a vasodilator and biological signaling molecule.[1, 2] Other RSS that contain sulfane sulfur (S^0) motifs, such as various polysulfides, participate in related biochemical processes, allow for the direct persulfidation of thiols, and enable crosstalk with NO through the formation of hybrid species like thionitrite/perthionitrite ($SNO^-/SSNO^-$).[3] Reductant labile S^0 sulfur pools are also involved in the formation of iron sulfur clusters, H_2S, polysulfides, and elemental sulfur (S_8).[4, 5] Interconversion of different S^0 motifs is also common, with anionic persulfides and tri/tetrasulfides generating S_8 upon decomposition.[5-7] Such chemistry provides an attractive hypothesis that S_8, which has a solubility in water (<20 nM) several orders of magnitude below other RSS,[8, 9] could be a potential storage source of S^0 prior to incorporation into other soluble S^0 species. Endogenous S_8 generation has been observed in several systems. For example, the Xun group recently demonstrated that bacteria with sulfide:quinone oxidoreductase (SQR) but no enzymes to further oxidize S^0 generated cytoplasmic sulfur globules.[10] Similar insoluble sulfur granules have also been observed in large sulfur bacteria (LSB) with the most notable example from the centimeter long bacteria *Candidatus Thiomargarita magnifica*.[11] S_8 is also an energy source for hyperthermophilic bacteria, such as *Staphylothermus marinus*, and crystallographic data shows that hydrophobic right-handed coiled coil nanotube (RHCCN) structures in these bacteria can bind S_8.[12]

Bridging the gap between S_8 and the soluble S^0 pool, we have recently investigated different approaches to solubilize S_8 in water and facilitate its reduction to H_2S with biological thiols (Figure 1a). For example, we showed that 50% wt-solutions of 2-hydroxypropyl β-cyclodextrin (2HPβ) can solubilize up to 2 mM S_8 in water.[13] Moreover, the solubilized S_8 can be efficiently reduced to H_2S by thiols and could efficiently sulfurate protein cysteine residues.[14] Using a related host-guest system, we also demonstrated that cucurbit[7]uril (CB[7]) can solubilize S_8 in water. Using this system, we established that the encapsulated S_8 is initially attacked by a thiol to generate a soluble S^0 carrier that is further reduced to polysulfides and ultimately H_2S by excess thiol.[15] Outside of host-guest chemistry, Steudel and co-workers also demonstrated that surfactants can increase the solubility of S_8 in water up to 0.103 mM in a chain length dependent manner, but the chemical accessibility of the solubilized S_8 was not investigated.[16] To further advance our understanding of modes of S_8 solubilization and activation, we demonstrate here that surfactants can not only solubilize S_8 in water but also promote the thiol-mediated reduction to H_2S. Moreover, we show that anionic and cationic surfactants differentially impact the speciation and equilibria of S^0 carriers, highlighting how different hydrophobic microenvironments interface with different RSS (Figure 1b).

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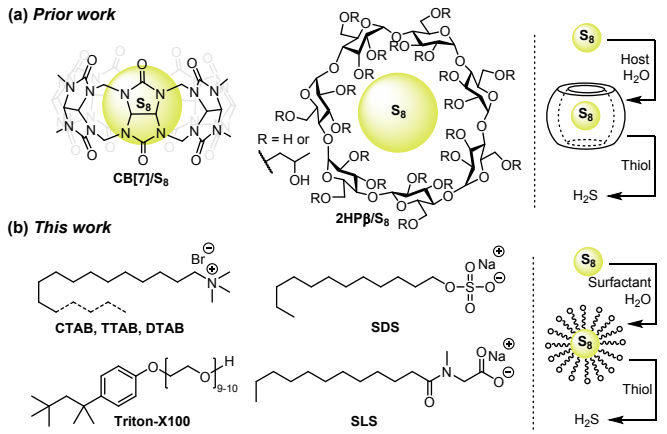

Figure 1. (a) Examples of prior work to activate S_8 in water toward reduction by thiols. (b) This work focuses on surfactants to solubilize and activate S_8 for reduction to H_2S by thiols.

Surfactants are long chained molecules bearing hydrophilic, often charged heads and lipophilic tails that aggregate in solution to form micelles with discrete hydrophobic interiors. Such surfactant micelles have been used previously to solubilize inorganic complexes, modify reaction kinetics, and model hydrophobic pockets present in cellular environments.[17-20] To investigate S_8 solubilization and activation by different surfactants, we stirred 100 mM solution of CTAB or SDS (10 mM PBS, pH = 7.4) with excess S_8 for two hours followed by filtration through 0.1 µm membrane filters to remove excess S_8. We then measured the resultant UV-vis absorbance to quantify the S_8 in solution (λ_{max} = 263 nm; ε = 6730 $M^{-1}cm^{-1}$) (Figure 2a).[21] As expected, we observed a significant increase in solubilized S_8 from each surfactant, corresponding to 150 µM and 65 µM for CTAB and SDS, respectively (Figure 2a). We next repeated these experiments with 100 mM Triton-X100, CTAB, TTAB, DTAB, SDS, and SLS, which is above the critical micelle concentration for each surfactant, to further investigate the role of alkyl chain length and charge on S_8 solubilization. Matching our expectation, increased S_8 solubilization was observed for longer chained CTAB and Triton-X100 when compared to shorter chained SDS, SLS, and DTAB (Figure 2b).

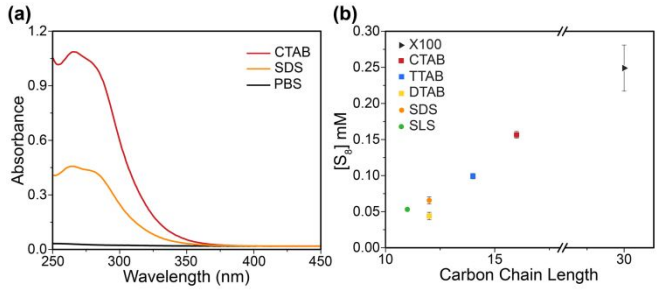

Figure 2 (a) UV-vis absorbance of S_8 in water with and without different surfactants. (b) Measured concentration of S_8 solubilized by different surfactants (100 mM) as a function of surfactant carbon chain length.

We next investigated whether the solubilized S_8 could be reduced to H_2S by treating each surfactant/S_8 system with cysteine, glutathione, homocysteine, and *N*-acetyl cysteine. We expected that the solubilized S_8 would be reduced by thiols, although the cationic versus anionic charge of micelles could differentially impact reactivity. To investigate this reactivity, we first monitored H_2S release from surfactant solutions containing 10 µM S_8 (80 µM S^0) treated with excess thiol (1 mM, 12.5 equiv.) using a Unisense SULF-500 H_2S sensitive electrode. We observed thiol-mediated H_2S release in each system, with H_2S rates depending on both thiol and surfactant identity (Figure 3a). For example, both the rate and overall efficiency of H_2S generation upon treatment with *N*-acetyl cysteine was greater with cationic surfactants (CTAB, DTAB, TTAB) than with anionic surfactants (SDS and SLS) (Figure 3a).[22] Despite these differences from surfactant and micelle charge, each surfactant that solubilized S_8 also promoted its reduction to H_2S when treated with various thiols (Figure 3b).

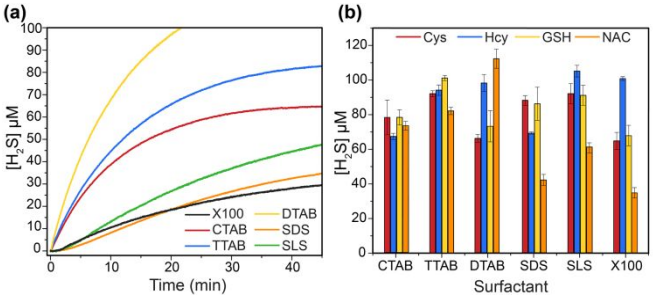

Figure 3. (a) H_2S release from 80 µM S^0 solubilized with different surfactants treated with N-acetyl cysteine (1 mM, 12.5 equiv.). (b) H_2S measured from S^0 (80 µM) solubilized with different surfactants (100 mM) and treated with different thiols (1 mM, 12.5 equiv.). Reported H_2S concentrations were measured after the release maximum (30-90 minutes).

We also investigated whether thiol pK_a impacted the rates of H_2S generation from different surfactants. For cationic surfactants, we did not observe a significant rate dependence on thiol pK_a. By contrast, we did observe a direct dependence on thiol pK_a for H_2S generation from anionic surfactants (Figure 4). This observed pK_a dependence matches what was observed in prior work with CB[7]/S_8 systems, in which the primary H_2S generating pathway relies on the reduction of soluble S^0 carriers in free solution by thiols.[15] In the anionic surfactant system, the observed thiol pK_a dependence means that the concentration of thiolate in solution directly impacts the rate of S_8 reduction, whereas this same dependence was not observed for cationic surfactants. The lack of pK_a dependence for the cationic system

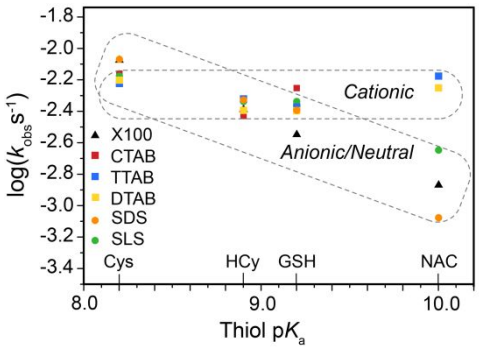

Figure 4. Comparison of the H_2S release rates from 10 µM S_8 (80 µM S^0) solubilized by each surfactant (100 mM, 10 mM PBS, pH=7.4, room temperature) treated with excess thiol (8 mM, 100 equiv.).

suggests that either the positive micelle charge may attract the negatively charged thiols or alternatively shift the effective pK_a of thiols within the local microenvironment.

Expanding the role of micelle charge on H_2S generation, we next investigated whether S^0-containing intermediates behave differently in anionic and cationic surfactants. The direct reduction of S_8 to H_2S requires 2 equiv. of thiol per S^0 atom and generates intermediate S^0 carriers in the forms of persulfides, hydropolysulfides, and inorganic polysulfides. We reasoned that such anionic intermediates may accumulate in cationic micelles, but be expelled from anionic micelles, due to the local electrostatic charge differences. To test this hypothesis, we varied the concentration of S^0 (50-250 μM) solubilized in CTAB and SDS, kept the Cys concentration constant (500 μM, 2-10 equiv. of thiol), and monitored H_2S levels in solution. For the cationic surfactant CTAB, we observed that the initial rates of H_2S production increased with increasing S^0 concentrations. Interestingly, for higher S^0 concentrations, this initial increase in H_2S formation was followed by rapid H_2S consumption (Figure 5a). We attribute this H_2S decrease to the reaction of H_2S with disulfides or related S^0-containing intermediates formed during the initial S_8 reduction, followed by accumulation of these species in the cationic micelle. By contrast, the anionic surfactant SDS showed increased H_2S generation with increasing S^0 concentrations without H_2S consumption at higher concentrations (Figure 5b). This behavior matches the expected behavior of reduction of soluble S^0 carriers in solution by thiols and suggests that these anionic intermediates are not accumulating within the anionic micelle. To further validate these results, we also treated S_8 solubilized in CTAB and SDS with NaSH and monitored H_2S levels. Under these conditions, we saw no change in H_2S levels in the presence of S_8 solubilized in SDS, but did see rapid consumption of H_2S for S_8 solubilized in CTAB (Figure 5c). These data further support that the cationic surfactant favors the accumulation and sequestration of anionic S^0 carriers.

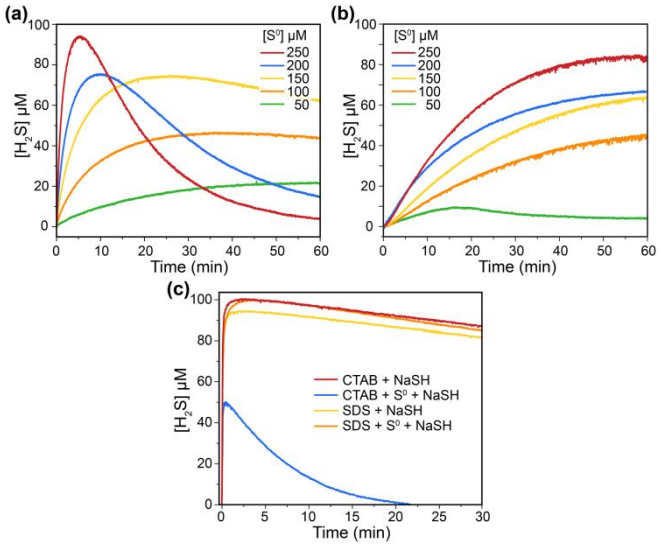

Figure 5. H_2S release from S^0 (50-250 μM) solubilized with (a) CTAB (100 mM) or (b) SDS (100 m) and treated with cysteine (500 μM, 2-10 equiv.). (c) H_2S levels in solution of CTAB or SDS (100 mM), with or without S_8 (250 μM) treated with NaSH (100 μM).

More broadly, the differential behavior of anionic and cationic surfactants toward solubilized S^0 carriers and H_2S highlights how the local charge environment can influence complex equilibria in the S^0 pool. For example, the observation that CTAB can decrease H_2S levels in solution when S^0 or oxidized sulfur species are present suggests that the cationic local environment can shift equilibria to favor accumulation of anionic species within the micelle. By contrast, SDS solubilized S_8 behaves analogously to the prior CB[7]/S_8 system in which the reduction chemistry to generate H_2S occurs in solution from soluble S^0 carriers. This behavior is further supported by the observed thiol pK_a dependence on H_2S generation rates for the anionic, but not cationic, surfactants (see Figure 4). Taken together, these data support the simplified model shown in Figure 6, in which cationic and anionic surfactants interact differently with the anionic S^0 carriers. Cationic surfactants accumulate anionic S^0 carriers, whereas anionic surfactants promote the formation of soluble S^0 carriers in free solution.

In summary, we have shown that common surfactants can solubilize S_8 in water, and that the S_8 can be reduced to H_2S by thiols. Cationic and anionic surfactants show different activity toward S_8 activation, with cationic species favoring the accumulation of anionic S^0 carriers from solution. Of specific relevance to the RSS field, we note that a variety of cationic surfactants are common additives (typically 100 μM – 5 mM) used with fluorescent probes for S^0 detection, and our data suggest that such additives may perturb the speciation of the S^0 landscape in solution.[23-25] More broadly, the ability of surfactants to solubilize S_8 and activate it toward reaction with thiols may have impacts in biological environments, in which hydrophobic motifs, such as lipid bilayers, may be able to transiently solubilize otherwise insoluble S^0 species prior to reincorporation into the soluble sulfane sulfur pool.

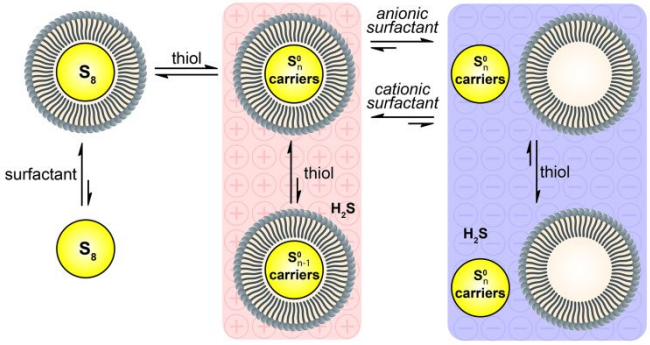

Figure 6. Simplified model of the differential activity of cationic and anionic surfactants toward solubilized S_8, anionic S^0 carriers, and H_2S.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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