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# Cationic, anionic, zwitterionic, and nonionic detergents differentially affect thermostability of pepper leaf proteins

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## ABSTRACT

Protein thermal stability is central to plant temperature stress responses as well as many practical enzyme applications. Considerable information has been acquired on interactions between specific pair-wise combinations of a protein and cosolute. However, little is known about interactions between complex protein mixtures, such as those found in plant cells, and cosolutes of various structural properties. Therefore, our objective was to determine the effects of four classes of detergents on solubility-based thermostability of pepper (*Capsicum annuum* L.) leaf proteins. The cationic detergent cetyltrimethylammonium bromide (CTAB) decreased protein thermostability with increasing CTAB concentration up to the concentration corresponding to the transition from CTAB monomers to micelles. However, higher concentrations of CTAB prevented turbidity and precipitation up to 100 °C. The anionic detergent sodium dodecyl sulfate (SDS) reduced pepper protein thermostability at low concentrations, but maintained solubility at higher concentrations. Unlike CTAB, the transition from destabilization to stabilization occurred over a lower SDS concentration range than the transition from monomers to micelles. In contrast with CTAB and SDS, the nonionic detergent polyoxyethylenesorbitan monolaurate (Tween 20) and the zwitterionic detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate (CHAPS) only destabilized pepper leaf proteins at elevated temperatures, approaching maxima at concentrations near the critical micellar concentrations. Results from this study support the hypothesis that destabilization results primarily from changes in hydrophobic interactions and stabilization occurs from charge repulsion limiting aggregation.



## KEYWORDS

*Capsicum annuum*;
Heat stress;
Protein stability;
Surfactant;
Thermotolerance.

## INTRODUCTION

Protein structure and function have been a focus of studies spanning fundamental mechanistic explorations to practical industrial applications. Changes in protein conformation and intermolecular interactions

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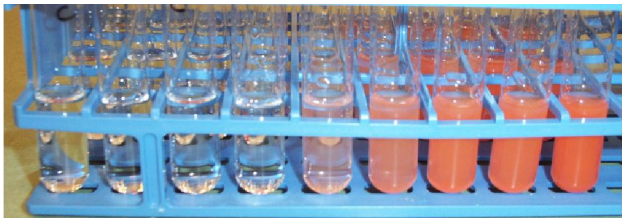
impact stress responses and disease progression in organisms, and affect reactions serving applications from biosensors to texture and quality of food products. Detailed information on structure and function of many individual proteins is available, but less is known about behavior of complex mixtures such as those found in plant and animal cells. One challenge presented by studying an individual protein is extension of results to other systems because proteins can vary widely in properties. While individual proteins are uniquely suited to many types of structural analyses, complex mixtures provide a more global basis to study physical changes in natural systems. Complex protein mixtures are well suited to studying environmental and chemical changes affecting intermolecular interactions that impact protein aggregation and loss of solubility. Protein folding anomalies, aggregation, and changes in solubility are fundamental to processes such as the heat shock response[1] and development of several neurodegenerative diseases[2].

Detergents are widely used in solubilization, purification, extraction and denaturation of proteins. Interactions between detergent and protein have been studied for many years and are not only of practical importance but also of theoretical significance, providing insight on protein denaturation and aggregation processes. Protein-detergent interactions are not only concentration dependent, but a function of the properties of the detergent and protein. The denaturing activity of ionic detergents is influenced by pH and ionic strength of the system. Nonionic detergents can maintain protein structure and function due to their neutral charges but may lower enzyme activity[3]. Zwitterionic detergents combine the features of ionic and nonionic molecules.

The nature of protein-detergent interactions can become more complex at higher detergent concentrations due to the formation of micelle structures when detergent concentration reaches the critical micellar concentration (cmc). In aqueous solution, micelle structure is thermodynamically favored by exposing the hydrophilic heads of the detergent molecules at the surface of the micelle and burying the hydrophobic tails inside the micelle to avoid contact with water. In a nonionic surfactant system, the major interactions between micelles are van der Waals attractive forces[4]. However, interactions between



**TOC Graphic : Illustrates change in properties as a detergent transitions from monomers to micelles. The procedure is a modified version of the dye solubilization technique**

charged micelles in an ionic system consist of electronic repulsion forces and solvation effects in addition to the van der Waals forces.

Detergent effects on protein thermostability have been studied using SDS (sodium dodecyl sulfate). Low concentrations of SDS had no influence on protein thermostability due to electrostatic binding with specific and limited sites of protein molecules[5]. When the concentration approached cmc, SDS decreased protein thermostability through hydrophobic interactions. However, when micelle structures formed at concentrations above cmc, protein molecules reportedly were captured and covered by micelle structures, increasing thermostability. An alternative model of protein-SDS micelle interactions is termed "necklace and bead"[6]. The two versions of this model include the protein wrapping around the micelles and hydrophobic portions of the protein passing through the micelle interiors.

Literature dealing with detergent-protein interactions has focused on individual proteins. Although many hypotheses have attempted to explain specific pair-wise combinations among various proteins and different types of detergents, divergent conclusions can be drawn when comparing proteins with different properties[7]. Furthermore, activity-based assays are specific to an arbitrarily chosen enzyme. One approach to deal with this dilemma is to evaluate solubility-based thermostability using extracts containing a mixture of proteins[8]. Mixtures can reflect average protein properties and the complexity of the cellular solution. Our primary objective was to determine the relationships between thermostability of pepper leaf proteins and detergents with different classes of head group over concentration ranges including both detergent monomers and micelles. A secondary objective was to compare a turbidity-based assay of protein thermostability with a colorimetric determination of soluble protein content.

## EXPERIMENTAL

### Plant culture and leaf extracts

'Early Calwonder' pepper (*Capsicum annuum* L.) plants were grown in 24-cm-diameter pots in a commercial potting mix (BM-1; Saint-Modeste, Que., Canada) amended with triple superphosphate (0.7 $g \cdot L^{-1}$), dolomite (3.6 $g \cdot L^{-1}$), Micromax (The Scotts Co., Marysville, OH) (0.6 $g \cdot L^{-1}$), and $KNO_3$ (0.6 $g \cdot L^{-1}$). Plants were maintained in a controlled-environment chamber (model PGW36; Conviron, Winnipeg, Man., Canada), at 24/20°C day/night temperatures with a 14 h photoperiod and a photosynthetic photon flux density at canopy height of about 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Plants were watered with soluble fertilizer (20N-8.6P-16.6K, Peters; The Scotts Co.) at 0.7 $g \cdot L^{-1}$ as needed based on media color and pot weight.

Fully expanded leaves were collected from 10-week-old plants. Paper towels wetted with deionized water were used to maintain leaf hydration during transfer from the growth chamber to the lab. Fifteen grams of leaves without the midrib were blended in a homogenization buffer consisting of 225 mL MES [2-(N-morpholino) ethanesulfonic acid] buffer [50 mM, pH=6.0, with 1 mM EDTA (ethylenediaminetetraacetic acid)] with 1.25 g PVPP (polyvinylpolypyrrolidone, Sigma-Aldrich Co., St. Louis, MO). The leaf slurry was filtered through Miracloth (Calbiochem, Madison, WI) and collected in a beaker containing an additional 1.25 g PVPP with 15 mL MES buffer. Slurries were stirred for 5 min, then the PVPP was allowed to settle for 2 min before decanting to a second beaker and settling for an additional 2 min before centrifugation. Leaf extract was generated by collecting the supernatants from centrifugation at 16k $g_n$ at 21ºC for 20 min. Extracts were mixed with an equal volume of MES buffer (control) or a 2x detergent solution prepared in MES buffer and stirred for 45 min. The extended stirring time was used because the highest concentrations of CTAB (2, 5 and 10 mM) were initially cloudy, but cleared over time with stirring at ambient temperature. After mixing, solution pH was adjusted as needed to 6.0 using NaOH except for the turbidity versus soluble protein content experiment where an aliquot was adjusted to pH 6.5. Averaged over all experiments, solutions contained 35.6 ± 4.6 mg protein per g fresh weight of leaf tissue based on a colorimetric assay with ovalbumin as the standard[9]. Detergents (SDS, CHAPS, Tween 20, and CTAB) were purchased from Sigma-Aldrich Co. Thermo Fisher Scientific (Waltham, MA) supplied EDTA and MES.

### Heat treatment

Treatment temperature ranges with 1ºC intervals yielding clear, cloudy, and precipitated samples were established in preliminary experiments. Three subsamples containing 3.4 mL solution from each treatment-temperature combination were pipetted to 16-mm diameter test tubes, except for the SDS experiment which employed two subsamples. A 21ºC treatment was included in each experiment as the control. Test tubes were held in water baths at target temperatures for 15 min, then placed in a water bath at 21 ºC for 4 h. In experiments with CTAB, a temperature treatment using a block heater at 100ºC was used in addition to water bath exposures for the 2 and 5 mM concentrations.

### Solubility measurements

A spectrophotometer (DU640B, Beckman Coulter Inc., Fullerton, CA ) was used to measure apparent absorbance at 540 nm ($AA_{540}$) 4 h after heat treatment. Un-heated control solutions (21ºC) from each treatment were filtered through 0.2 μm polyethersulfone membrane syringe filters for use as blanks. As treatment temperatures increased, light scattering by aggregated proteins increased $AA_{540}$ values until a maximum value ($AA_{540\text{-}MAX}$) was reached. Further temperature increases yielded $AA_{540}$ values similar to controls due to precipitate formation. Only the clear solution over precipitates was sampled in experimental units with insoluble proteins.

### CMC measurement

Preliminary experiments indicated that a single procedure to measure cmc was not applicable to all detergent classes. The cmc value of CTAB in pepper leaf protein solutions was measured using a modification of the dye solubility procedure[10]. Approximately 2 mg cadmium red light paint (Duro, Chicago, IL) was added to the bottom of 16-mm diameter test tubes using a wooden rod. Five mL CTAB at 0, 0.005, 0.05, 0.1, 0.5, 1, 2, 5 or 10 mM in MES buffer was added and vortexed every 10 min for 2 h, then per-

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cent transmission was measured at 540 nm. Solutions became turbid from solubilization of the paint carrier at cmc. For SDS, the absorbance of chlorophyll in the SDS-extract mixture was measured at wavelengths of 415, 437, 671, and 679 nm to detect spectral shifts associated with micelle formation[11]. The cmc for SDS was also estimated using the paint solubility assay. Eosin Y (Coleman & Bell Co., Norwood, MA) at a final concentration of 4.75 µM was used to measure cmc values for CHAPS and Tween 20[12]. Spectral shifts were detected by measuring absorbance at 518 and 528.5 nm.

### Data analyses

Each experiment was conducted with five independent replications (dates) with three subsamples per treatment combination in each replication, except the SDS experiment which had two subsamples. Analysis of variance was conducted using PROC GLM (SAS Institute, Cary, NC) to analyze treatment effects with treatment by date as the error term, and the interaction effect between treatment and temperature with the error term of treatment by temperature by date for $AA_{540}$ response data. A highly significant ($P \leq 0.01$) treatment by temperature interaction was observed in all experiments. A separate analysis of variance was then performed for treatment and interaction effects using PROC GLM for the $AA_{540\text{-}MAX}$ response variable. Duncan's new multiple range test was conducted as appropriate to group treatment means using treatment by date as the error term at the critical value of $P \leq 0.05$. Midpoints of cmc response variables versus detergent concentration were estimated using PROC NLIN. Transmission values were transformed ($100\text{-}T_{540}$) to convert to a sigmoidal response curve with increasing values in the paint solubilization assay. Peak ratios were used in spectral shift assays.

## RESULTS

### Turbidity versus soluble protein at two pH values

At pH 6.0, turbidity measured by $AA_{540}$ increased with increasing temperature to 50°C (Figure 1). At temperatures higher than 50 °C, $AA_{540}$ decreased markedly, corresponding with the formation of precipitates. The soluble protein concentration based on the Bradford[9] colorimetric assay remained relatively constant over the temperature range from 21 to 48°C (Figure 1). The concentration of soluble protein decreased sharply at temperatures higher than 50 °C. At pH 6.5, $AA_{540}$ increased with increasing temperature to 56°C. At temperatures higher than 56°C, $AA_{540}$ decreased sharply as precipitates formed. The soluble protein content based on the colorimetric assay remained relatively constant over the temperature range from 21 to 54°C, then decreased markedly at temperatures higher than 54°C (Figure 1).

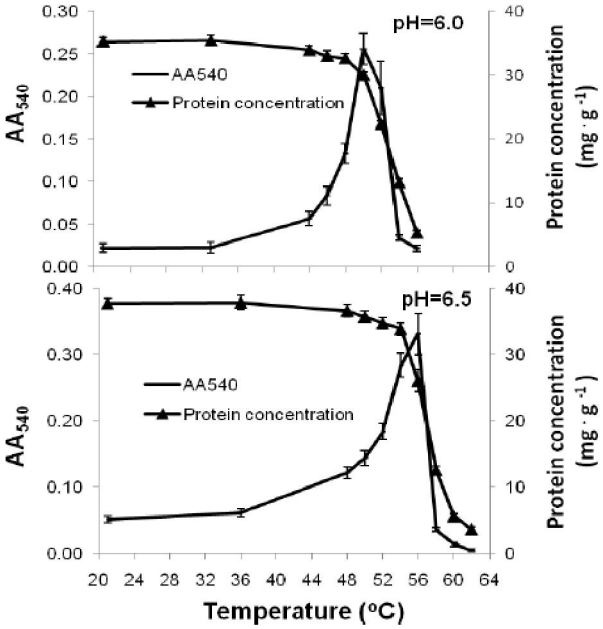


**Figure 1 : Apparent absorbance at 540 nm ($AA_{540}$) and Bradford-quantified soluble protein content versus temperature for pepper leaf extracts at pH 6.0 and 6.5**

### Cationic CTAB

Concentration-dependent effects of CTAB on pepper leaf protein thermostability were exhibited. Proteins were destabilized as CTAB concentrations increased to 1 mM (TABLE 1). Compared to the control, CTAB at 0.05 mM decreased $AA_{540\text{-}MAX}$ by 6.8ºC. At CTAB concentrations of 0.5 and 1 mM, protein precipitated at 21ºC, the lowest treatment temperature examined. However, no turbidity increase or precipitation was observed following 100°C exposures at CTAB concentrations of 2 and 5 mM, indicating a strong stabilizing effect at these concentrations. The transition from strongly destabilizing to extreme stabilization was between the concentrations of 1 and 2 mM, which corresponded with CTAB's cmc value of $0.9 \pm 0.2$ mM in

this system. Even though pepper leaf proteins were boiling stable in the higher CTAB concentrations, enzymatic activity based on catalase (EC 1.11.1.6) activity was lost (data not presented).

**TABLE 1 : Apparent absorbance maxima temperatures for pepper leaf extracts containing cetyltrimethylammonium bromide (CTAB)**

| CTAB (mM) | $AA_{540\text{-}max}$ temperature (°C) |
|---|---|
| 0 | 52.4 ± 0.2 a[Z] |
| 0.005 | 53.0 ± 0.3 a |
| 0.05 | 45.6 ± 0.4 b |
| 0.5 | Y[Y] |
| 1 | Y |
| 2 | X[X] |
| 5 | X |

**[Z]Means in the column with the same letter are not significantly different by Duncan's new multiple range test with P ≤ 0.05. [Y]Y= precipitation was observed at all temperatures, including the control temperature of 21°C, the lowest temperature in this experiment. [X]X= no turbidity or precipitation formed at all temperatures examined, including 100°C**

## Anionic SDS

Dual effects of SDS on pepper leaf protein thermostability were exhibited. Leaf proteins became less stable as SDS concentrations increased from 0.005 to 0.15 mM (Figure 2). The greatest destabilizing effect was observed at 0.15 mM with a mean $AA_{540\text{-}MAX}$ temperature 3.6ºC lower than the control. However, a stabilizing effect was observed at SDS concentrations from 0.25 to 0.35 mM. Proteins were 11ºC more thermostable than controls at 0.35 mM, the highest SDS concentration examined. Micelles formed at higher SDS concentrations than observed for the transition from destabilizing to stabilizing proteins. The cmc estimates for SDS were 2.3 mM based on the paint solubilization assay, 2.8 mM based on the chlorophyll absorption shift at 437/415 nm, and 2.2 mM based on the chlorophyll absorption shift at 679/671 nm (Figure 3).

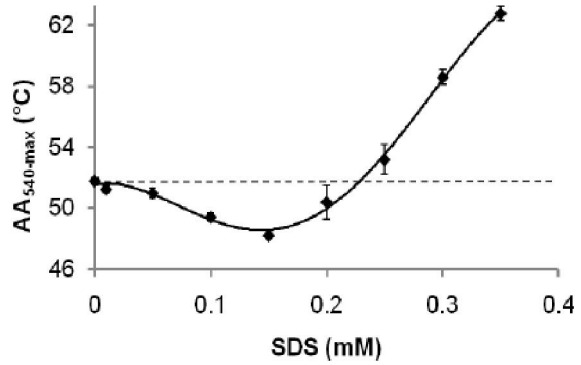


**Figure 2 : Dose- response of SDS on thermostability of pepper leaf proteins**

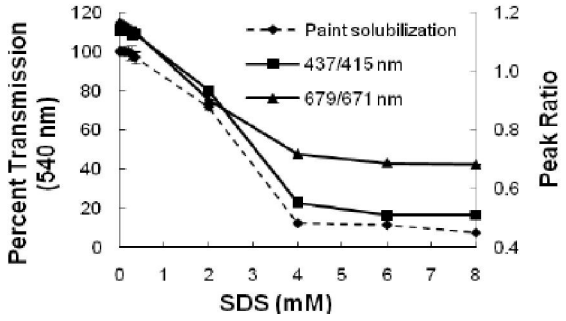


**Figure 3 : Effects of SDS concentration on paint solubilization (percent transmission at 540 nm) and peak ratios for chlorophyll spectral shifts (437/415 and 679/671 nm)**

## Nonionic Tween 20

Tween 20 did not stabilize pepper leaf proteins (Figure 4). Destabilization was observed at Tween 20 concentrations of 1 and 10 mM, with no significant differences between 1 and 10 mM treatments. The cmc for Tween 20 was 0.6 ± 0.3 mM.

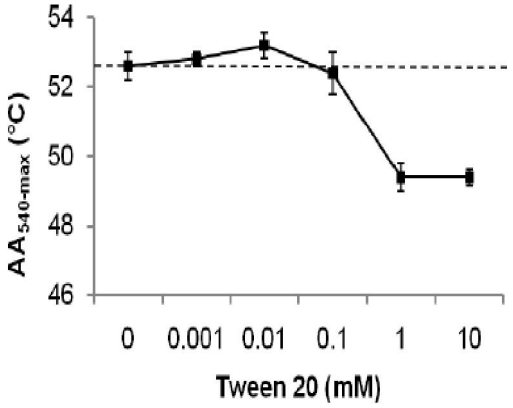


**Figure 4 : Dose- response of Tween 20 on thermostability of pepper leaf proteins**

## Zwitterionic CHAPS

Pepper leaf proteins were destabilized by CHAPS at concentrations greater than 1 mM (Figure 5). As CHAPS concentration increased from 1 to 5 mM, $AA_{540\text{-}MAX}$ temperatures decreased, reaching a minimum 5.6ºC lower than the control at 5 mM. There were no significant differences between 3 mM and 5 mM treatments. The cmc for CHAPS was 5.5 ± 0.1 mM.

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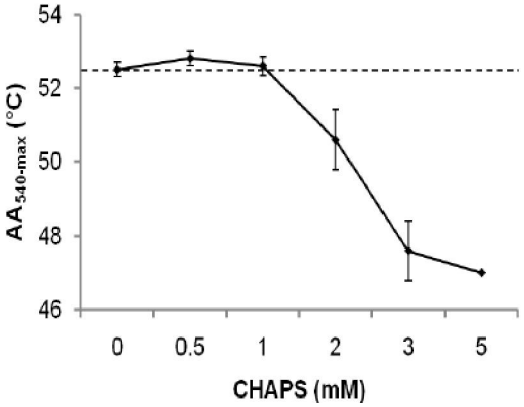


**Figure 5 : Dose- response of CHAPS on thermostability of pepper leaf proteins**

## DISCUSSION

### Turbidity versus soluble protein at two pH values

Turbidity changes determined by $AA_{540}$ measurement were consistent with changes in the soluble protein content measured by Bradford quantification. As the turbidity of the solution increased due to the formation of small aggregates, the soluble protein content measured by Bradford quantification remained fairly constant. Both turbidity and colorimetrically-determined soluble protein content decreased when precipitates formed due to severe heat aggregation. The close correspondence between the methods strongly suggests that changes in protein structure and solubility are primarily responsible for turbidity changes in the plant extract and further validates use of $AA_{540}$ to measure protein thermostability. It is more convenient than colorimetric assays and provides additional information on the formation of small aggregates. Unlike using circular dichroism spectroscopy to measure changes in protein conformation, $AA_{540}$ does not require expensive equipment and is well suited to complex mixtures.

It is well known that pH affects protein stability, primarily through changes in electrostatic interactions. Therefore, similar shifts in both methods should be exhibited at a higher pH if they represent the same phenomenon. The correspondence between changes in turbidity and soluble protein content was exhibited at both pH 6.0 and 6.5. Within the range of 6.0 to 6.5, the higher pH environment had a greater ability to maintain protein solubility, in agreement with previous work with thermostability of leaf proteins[13]. Results were consistent with more negative charges at the higher pH introducing stronger repulsive forces between proteins and attenuating high temperature aggregation.

Irreversible high-temperature-induced changes in protein conformation can also be monitored by residual activity of enzymes after return to ambient temperature. Recognizing that choice of a particular enzyme is arbitrary in that enzymes can vary widely in temperature dependency and stability, we selected the catalase assay to determine whether responses of an activity-based assay to pH corresponded with results from solubility-based assays. Both types of protein thermostability assays showed an increase in stability at pH 6.5, compared with pH 6.0 (data not presented).

### Cationic CTAB

Concentration-dependent effects of CTAB on pepper leaf protein thermostability were observed over the range from 5 μm to 5 mM. Destabilization occurred up to 1 mM with concentrations of 0.5 and 1 mM causing aggregation of un-heated controls. However, 2 and 5 mM CTAB strongly stabilized proteins against aggregation and precipitation. Since the cmc (0.9 mM) was close to the transition concentration range from destabilizing to stabilizing, it is likely that CTAB monomers destabilize pepper leaf proteins and CTAB micelles act as stabilizers. Consistent with our results, a previous study found that micromolar CTAB concentrations precipitated tobacco mosaic virus coat protein at room temperature[14]. The authors proposed that positively charged CTAB head groups bound to negatively charged sites on the protein, allowing the exposed CTAB tail to promote hydrophobic interactions leading to aggregation. It is also possible that hydrophobic tails of some monomers penetrated into the hydrophobic protein core, disrupting protein structure and lowering thermostability.

Expanding models of protein-CTAB interactions to higher concentrations requires taking into consideration the formation of micelles. Micelle structure shields the hydrophobic tails of CTAB monomers and attenuates hydrophobic aggregations among CTAB-protein complexes. Depending on relative size and abundance of protein molecules and micellar cavities, as well as protein hydrophobicity, proteins could be encapsulated within or partially embedded within a micelle, or CTAB

micelle structures could bind to protein surfaces via electrostatic interactions. If micelles bind to surfaces of large proteins, repulsion forces between positively charged micelles must outweigh attractive forces with residual negative charges on proteins, possibly through steric hindrance, or result in charge neutralization. If proteins are encapsulated, it appears likely that at least partial protein unfolding would be favored in the hydrophobic core of a micelle. Protein stabilization in cavities via a decrease in conformational mobility has also been suggested[15]. Regardless of whether micelles interact with proteins through electrostatic interactions or exist as a shell, repulsion from the exposed positive charges of CTAB would inhibit aggregation.

**Anionic SDS**

Sodium dodecyl sulfate has been used extensively to maintain protein solubility in many applications, including SDS-gel electrophoresis[16]. Although enzymes frequently lose activity, precipitation is prevented by high SDS concentrations. Pepper leaf proteins were destabilized at low SDS concentrations, but remained soluble to higher temperatures when SDS was present at greater than 0.25 mM. Concentration-dependent effects of SDS on protein structure have also been reported for cytochrome C[6], lysozyme[5], and cutinase[17]. Corresponding results were obtained with an SDS/Tween 20 combination in studies of molecular interactions in immunoassays[15]. A transition was observed for immunoglobulin G thermostability with a cut-off concentration much lower than the cmc. Similarly, inhibition of tobacco mosaic virus coat protein aggregation was first detected at 57.5 μM SDS[18]. Vermeer and Norde[15] interpreted the phenomenon as a dominance shift between electrostatic interactions and hydrophobic interactions of the detergent-protein complexes. Since electrostatic interactions have higher affinity than hydrophobic interactions, initial binding is between ionic groups[19]. The negatively charged heads of SDS monomers bind with the positively charged residues of proteins, exposing the hydrophobic tail. Driven by hydrophobic interactions, the exposed hydrophobic surfaces are favored to aggregate in order to lower the free energy in an aqueous solvent. The hydrophobic tails of some detergent monomers could also penetrate into the protein hydrophobic core, deforming protein structure. At higher SDS concentrations (yet below cmc) additional detergent molecules would be available to bind to exposed hydrophobic patches, preventing intermolecular binding. In the "necklace and bead" models, partially unfolded proteins interact with micelle-like detergent structures that are stabilized at sub-micellar concentrations through interactions with portions of the protein molecules[6]. At low SDS concentrations the interactions are electrostatic at the protein surface. Exposed hydrophobic portions of the protein can then participate in hydrophobic interactions with other proteins leading to aggregation. At higher SDS concentrations, hydrophobic patches of proteins are located in the interior of detergent structures, decreasing opportunities for aggregation. It is also possible that a mixed model can be applied with maximal stabilization resulting from both shielding of hydrophobic patches and electrostatic repulsion, a consequence of protein molecules both wrapping around and passing through detergent clusters. Alternatively, the stabilizing effect could be attributed to an SDS monolayer accumulating on the protein surface, limiting unfolding space by a confined environment.

Although both SDS and CTAB are ionic detergents and capable of binding to charged residues, the dose-response patterns are different with respect to micelle formation and protein thermostability. The basis for the difference may lie in a greater number of negatively charged residues compared with amino acid side chains carrying a positive charge, consistent with stabilization of pepper leaf proteins at higher pH[13].

**Nonionic tween 20**

The non-ionic detergent Tween 20 only destabilized pepper leaf proteins, reaching a maximum near the cmc. The dose-response behavior may result from saturation of the interior space of protein molecules by detergent insertion. When no more Tween 20 monomers can be loaded inside a protein, the preferred conformation is to leave the hydrophobic tails of the Tween 20 monomer outside the protein molecule with the head binding to the hydrophilic surface of the protein. Exposure of hydrophobic tails is thermodynamically disfavored, driving aggregation of Tween 20-protein com-

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plexes. The destabilizing effect reached a plateau at 1 mM Tween 20, corresponding with the cmc for this system (0.9 mM). It is likely that once micelles form, attraction between polar head groups favors aggregation. Electrostatic repulsion does not occur since there are no charges on the head of Tween 20.

**Zwitterionic CHAPS**

As a charged detergent, interactions between CHAPS monomers and protein molecules can consist of both hydrophobic and electrostatic interactions. However, different from either anionic or cationic detergents, both positive and negative charges on the surface of proteins can be neutralized. Although electrostatic interactions can be strong between the charged head of CHAPS and proteins, zwitterionic detergents are considered intermediate in properties between ionic and nonionic detergents. Although zwitterionic detergents can prevent aggregation at concentrations near cmc[20], an initial report of protein destabilization by a zwitterionic detergent was made for cutinase[7]. Our results with CHAPS and pepper leaf proteins support the finding of protein destabilization by a detergent carrying both positive and negative charges. Accumulation of CHAPS monomers at the exterior of the protein promoted aggregation between protein-detergent complexes. Although concentrations higher than 5 mM were not examined, it appears that protein destabilization reached a maximum near the cmc for CHAPS (4.4 mM). It is likely that electrostatic repulsion fails to be established, but hydrophobic aggregation is attenuated. As a result, the transformation from monomeric CHAPS to micelles does not lead to further destabilization of proteins.

## ABBREVIATIONS

$AA_{540\text{-}MAX}$, maximum apparent absorbance at 540 nm; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate; CMC, critical micellar concentration; CTAB, cetyltrimethylammonium bromide; SDS, sodium dodecyl sulfate; Tween 20, polyoxyethylenesorbitan monolaurate.

## CONCLUSIONS

The effects of detergents on protein thermostability varied with detergent class, and with the same detergent at different concentrations. Nonionic Tween 20 and zwitterionic CHAPS had similar effects on pepper leaf protein thermostability. Both detergents destabilized proteins with maximum destabilization close to the cmc. Cationic CTAB and anionic SDS destabilized proteins at low concentrations, but increased thermostability at higher concentrations. The transition from destabilization to stabilization occurred near the cmc for CTAB, but was at sub-micellar concentrations for SDS. Similar results were reported for denaturation of α-lactalbumin at 22 to 25°C[21]. Sub-micellar concentrations of anionic and cationic detergents denatured α-lactalbumin, but nonionic and zwitterionic detergents interacted weakly at concentrations below the cmc, "priming" the protein for structural changes at the cmc.

Differences between detergent classes can be explained by differences in binding behavior. Destabilization can result from hydrophobic interactions and stabilization occurs primarily from charge repulsion limiting aggregation. While denaturation from partial unfolding and aggregation can be related processes, denatured proteins can be strongly stabilized against aggregation and precipitation. Since proteins are also amphipathic molecules, protein-detergent interactions can provide additional insight on protein-protein interactions in unfavorable environments. The present study extends knowledge of protein-detergent interactions at elevated temperatures across all four polar head group types and to complex protein mixtures such as would be found in plant protoplasts.

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