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A Proposed Mechanism for Detergent-Assisted Foam Fractionation of Lysozyme and Cellulase Restored With β-Cyclodextrin

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Abstract

Foam fractionation by itself cannot effectively concentrate hydrophilic proteins such as lysozyme and cellulase. However, the addition of a detergent to a protein solution can increase the foam volume, and thus, the performance of the foam fractionation process. In this article, we propose a possible protein concentration mechanism of this detergent-assisted foam fractionation: A detergent binds to an oppositely charged protein, followed by the detergent–protein complex being adsorbed onto a bubble during aeration. The formation of this complex is inferred by a decrease in surface tension of the detergent–protein solution. The surface tension of a solution with the complex is lower than the surface tension of a protein or a detergent solution alone. The detergent can then be stripped from the adsorbed protein, such as cellulase, by an artificial chaperone such as β-cyclodextrin. Stripping the detergent from the protein allows the protein to return to its original conformation and to potentially retain all of its original activity following the foam fractionation process. Low-cost alternatives to β-cyclodextrin such as corn dextrin were tested experimentally to restore the protein activity through detergent stripping, but without success.

Index Entries: Artificial chaperones; detergent stripping; protein refolding; protein renaturation; surface tension.

Introduction

Foam fractionation has the potential to be an effective low-cost protein separation and concentration process *(1–5)*. Foam fractionation has a much lower cost than traditional protein separation techniques such as chromatography, ion exchange, electrophoresis, and ultrafiltration *(4,5)*. Selectivity can be imparted to the foam fractionation technique by varying the pH of the foamed solution *(6)*, the airflow rate used in the foaming, or the initial liquid volume. The low cost of foam fractionation suggests that the process could be used in the pharmaceutical industry wherein therapeutic protein drugs

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need cost-effective purification and separation techniques, as downstream processing is one of the major costs in biopharmaceuticals production *(7)*. Foam fractionation is a versatile process that can be applied to other potentially industrial scale processes, such as, concentrating laccase C, a lignin-degrading enzyme that is used in chlorine-free bleaching processes *(8,9)*. Nonetheless, foam fractionation does not work well with hydrophilic proteins such as lysozyme, because such proteins often do not foam when aerated at low concentration *(10)*. One way to modify foam fractionation to concentrate hydrophilic proteins is to add a detergent to increase the foam volume. This has been demonstrated in previous studies wherein it has been observed that the addition of a detergent can enhance the concentration of hydrophilic proteins (like lysozyme and cellulase) in foam fractionation processes *(11–13)*. Unfortunately, the protein may be denatured during the process, but it is possible to restore some of the lost activity by adding cyclodextrin. The mechanism of this detergent-assisted foam fractionation has apparently not been explored; therefore, a molecular mechanism to model this process is proposed in this article for possible use in future process improvement studies.

Although cyclodextrins have been used successfully to restore activity to enzymes they are relatively expensive, particularly when used with industrial enzymes like cellulase, which can currently sell for just a few dollars per kilogram. It is the cyclodextrin's hydrophobic cavity and the inclusion complexes it forms that allow it to strip away the charged surfactant and restore the activity *(7)*. Other starches and dextrins may have the potential to remove the detergent from the cellulase if they are able to form a structure similar to the hydrophobic cavity of the cyclodextrin. Long linear dextrins may be able to form such hydrophobic chambers in transient states, capable of removing the detergent from the enzyme. However, little seems to be known about substituting other dextrins for cyclodextrins in order to reduce the cost of enzyme restoration *(7)*.

In order to concentrate a protein solution using a foam fractionation process, the protein must first adsorb onto a foamed air (or other gas) bubble surface. A hydrophilic protein like lysozyme is not likely to adsorb because it prefers to stay in aqueous solution. Without that adsorption step, the concentration of the resulting product "foamate" will be the same as the initial concentration. However, by adding a detergent, which forms a complex with the hydrophilic protein, the hydrophilic part of the detergent likely binds with the hydrophilic part of that protein. The hydrophobic part of the detergent is then free to be adsorbed on the bubble surface and is then free to be carried along with the attached protein out into the foam phase. At the same time, the water will drain as the foam rises to the top of the column making the protein even more concentrated in the foamate.

Materials and Methods

Cellulase from *Trichoderma reesei*, lysozyme from chicken egg white, lyophilized cells of *Micrococcus lysodeikticus*, sodium dodecylsulfate (SDS), Pluronic F-68, and 3,5- dinitrosalicylic acid (DNS) were purchased from Sigma (St. Louis, MO). Cetyltrimethylammonium bromide (CTAB) was purchased from Fluka (Switzerland). Starch soluble, potato starch, corn starch, Whatman filter paper No. 1,3-cyclodextrin, bicinchoninic acid (BCA) assay kit, and 96-well microplate were purchased from Fisher Scientific (Pittsburgh, PA). The artificial sweetener stevioside (Stevia) was provided by Flavio F. DeMoraes from Maringa, Brazil. 10 m*M* pH 5.0 phosphate buffer prepared at time of use or deionized water was used as a solvent in the cellulase experiments *(14)*, and 10 m*M*, pH 8.5 Tris-HCl buffer was used in the lysozyme experiment *(15)*. The components used in preparing these buffer solutions were purchased from Sigma. All data except for the surface tension data and refolding with linear dextrins data are taken from earlier work *(11)*.

Foam Fractionation

Semibatch foam fractionation experiments are carried out in a small custom made (with stopcock at the bottom) glass column (2×10 cm^2). The schematic drawing of the apparatus is shown in Fig. 1, from a previous publication *(16)*. Twelve milliliters of protein solution is added to the column and medical grade air from a compressed gas cylinder is introduced (at a selected rate) through a fritted disc sparger (pore size 40–60 μm) imbedded at the bottom of the column. Water loss in the effluent air stream is minimized by humidifying the air before it enters the column. Air is allowed to continue to flow into the column at rates of 4, 8, and 12 mL/min until no more foam is generated. The produced foam is allowed to continuously collapse into a liquid product (the foamate in the overhead foam collector). The foamate volume is measured in a graduated cylinder. Each experimental condition is repeated in triplicate.

Renaturation of Cellulase After Foam Fractionation

After foam fractionation of a cellulase and CTAB mixture, 350 μL of collected foamate is diluted with 150 μL of 13 m*M* β-cyclodextrin solution or varying concentrations of corn dextrin solution, potato starch solution, Stevia (a Brazilian sugar substitute) solution, or starch soluble solution. The resulting mixture is stored overnight before checking for its activity, both before and after addition of β-cyclodextrin and the other potential refolding solutions. The filter paper activity test *(17)* is used to determine the cellulase activity. The DNS assay *(18)* is used to measure the amount of sugar produced in that test.

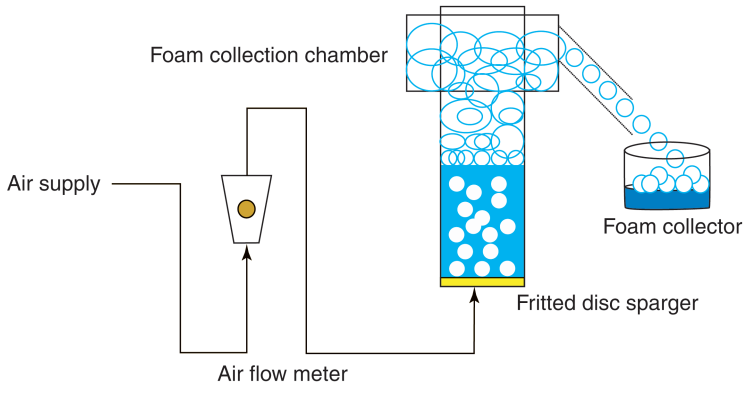

Fig. 1. Schematic drawing of the foam fractionation apparatus.

BCA Assay for Protein Concentration

Twenty mililiters of collected cellulase samples are put into a 96-well microplate and the analysis is repeated in triplicate. Then, 180 µL of BCA reagent is added to each well-plate. The microplate is scanned after 30 min for the determination of absorbance at 562 nm *(19)*. For lysozyme, 20 µL of the sample is added to a well in a 96-well microplate. 180 mL of BCA reagent is then also added to each well and again carried out in triplicate. The microplate is incubated at 37°C for 2 h before being read by a Bio-Tek µQuant plate reader at 562 nm. Longer incubation times and higher temperatures are used to increase the light absorbance signal from the BCA assay, when necessary.

Lysozyme Activity Assay

The protocol for this assay is taken from the Worthington Enzyme Manual *(20)*. Lyophilized *M. lysodeikticus* cells of 9.0 mg are first diluted in 25 mL of 0.1 *M* potassium phosphate buffer at pH 7.0. Then, after dissolving the *M. lysodeikticus* cells, the buffer solution is added to bring the final volume to 30 mL. Ten microliters of the samples are added to a 96-well microplate. BCA reagent of 290 µL are then added to each well. The assay is carried out in triplicate. The change in absorbance at 450 nm is scanned every minute for 5 min using the Bio-Tek µQuant plate reader. The activity of the substance is measured in units/mL and activity enrichment (AE) is defined as the activity per unit volume of the foamate divided by the initial activity per unit volume of the solution before foaming.

Surface Tension Measurement

The surface tension of solutions is determined by the Wilhelmy Plate method using a Sigma 70 tensiometer from KSV Instrument Ltd. (Helsinki, Finland). The automated tensiometer apparatus determines surface tension

by slowly dipping a platinum-iridium plate into the surface of the liquid solution. Then, the microbalance inside the tensiometer measures the maximum force needed to pull the plate away from the liquid surface. The accompanying computer calculates the liquid surface tension based on the force measured. Twenty milliliters of liquid sample is placed into a glass cylinder, which in turn, is placed within the KSV Sigma 70 tensiometer. After pressing the start button on the computer interface, the surface tension of the liquid solution is measured five times and the results are then averaged.

The surface tension of six separate systems is measured to determine the critical micelle concentration (CMC) of various surfactants in different solvents. The CMC is the surfactant concentration wherein any additional amount of surfactant does not change the surface tension of the solution. First a 10.0 mg/mL SDS solution is added incrementally to deionized water, and the resulting surface tension is measured at various concentrations to determine the CMC of SDS in water. Then, a 10.0 mg/mL SDS solution is added incrementally to a 75.0 mg/L lysozyme solution to determine the CMC of SDS in 75.0 mg/L lysozyme solution. In addition, lysozyme is also incrementally added to deionized water to determine the CMC of lysozyme in water. Next, 1.0 mg/mL CTAB solution is added incrementally to deionized water to determine the CMC of CTAB in water. A 1.0 mg/mL CTAB solution is added incrementally to 200.0 mg/L cellulase solution to determine the CMC of CTAB in 200.0 mg/L cellulase solution. Finally, cellulase is also added to deionized water to determine the CMC of cellulase in water.

Results and Discussion

Summary of Detergent-Assisted Foam Fractionation of Lysozyme

Varley and Ball *(10)* tried to use foam fractionation to concentrate a lysozyme solution and reported that in order to foam lysozyme, a high-protein concentration of 2200.0 mg/L needed to be used. With this high lysozyme concentration, they obtained an enrichment ratio (ER) as high as 1.2 with a 0.02 protein mass recovery (MR). They also retained about 87% of the initial activity. The use of an SDS-assisted foam fractionation makes it possible to foam lysozyme at a lower concentration, which in the presented data, can result in a much larger ER (3.3). Results from the literature indicates that anionic surfactants such as SDS bind strongly with proteins and form protein–surfactant complexes; cationic surfactants (e.g., CTAB) on the other hand, have less tendency to interact with proteins, and non-ionic surfactants (e.g., Pluronic F-68) weakly bind to proteins *(21–23)*. For example, lysozyme at pH 8.5, the condition used for foaming, is cationically charged (on average) suggesting its strong desire to bind with anionic surfactants such as SDS *(21–23)*. It has been determined that a complex of lysozyme–SDS is more surface active than lysozyme or SDS alone *(24)*. Our direct surface tension measurements indicate that this enhanced

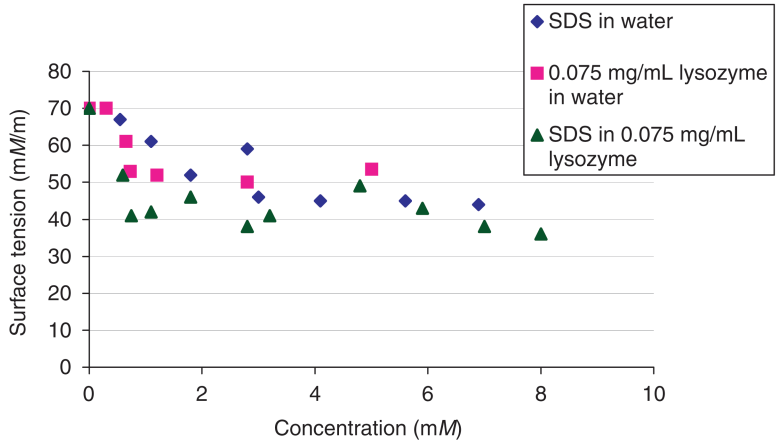

Fig. 2. Surface Tension measurements of SDS, lysozyme, and SDS–lysozyme complex. The surface tension is measured by the Sigma 70 (tensiometer) using the Wilhelmy Plate Method.

surface activity (Fig. 2). Thus, it is possible that the complex may foam better than its individual parts, which in fact is the case, resulting in better enrichment and recovery. From Fig. 2, the CMC of SDS in water is seen to be around 4 m*M*, the CMC of SDS in 75.0 mg/L lysozyme solution is around 1.5 m*M*, and the CMC for lysozyme in water is around 3 m*M*. Thus, the SDS-assisted foam fractionation of lysozyme (200.0 mg/L SDS = 0.7 m*M*) is operated below the CMC. Operating below the CMC is important to avoid the formation of miscelles. SDS normally binds with a protein by electrostatic interaction with the positively charged groups of amino acids of that protein *(25)*, so that the complex is likely to have strong electrostatic binding. Lysozyme does not show any loss of activity during foam fractionation probably because of the fact that lysozyme contains four disulfide bonds, which may make the lysozyme more resistant to structural change. Moreover, a minor change in structure as noted from CD measurements, is probably not enough to change lysozyme activity *(26,27)*. It is reported in the literature that without reducing the four disulfide bonds into lysozyme, a rapid dilution can effectively refold the denatured lysozyme into its native state *(28)*. In this foaming process, no reducing agent was used; therefore, the four disulfide bonds are likely to remain intact, making it more difficult for lysozyme to lose its enzymatic activity. A schematic for the SDS- and lysozyme-binding process is pictured in Fig. 3.

Of all three detergents tested (CTAB, Pluronic F-68, and SDS), addition of SDS yields the highest enrichment and recovery *(29)*. Addition of SDS to the foam fractionation of lysozyme improves both the enrichment and recovery when compared with foam fractionation of lysozyme without any additional detergent *(10)*. Although there is a slight change in

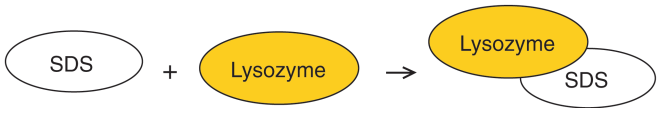

Fig. 3. SDS binds with Lysozyme. Owing to the four disulfide bonds lysozyme is resistant to structural changes caused by this binding. This means that the lysozyme activity remains intact in the binding process.

lysozyme secondary structure, there is no loss of lysozyme activity as a result of SDS-assisted foam fractionation. The SDS–lysozyme complex is more surface active than SDS or lysozyme alone because of the greater depression of surface tension with the complex than with either of the components. Operating SDS-assisted lysozyme foam fractionation at an airflow rate of 4 mL/min gives the best result because it yields the highest ER and leads to the recovery of about 86% of the initial enzyme, whereas operating the foam column at the other two airflow rates (8 or 12 mL/min) leads to 9% higher recovery and an enrichment loss of less than half.

Summary of Detergent-Assisted Foam Fractionation of Cellulase

Without the addition of β-cyclodextrin solution, operating with additional 100.0 mg/L SDS at 12 mL/min is probably the best condition among the three detergents tested. This condition provides the lowest loss of activity. Although Pluronic F-68 does not lead to any loss of activity, it does not increase the protein concentration in the foamate, so it is not beneficial to this process. With the addition of β-cyclodextrin, CTAB is probably the best detergent among the three detergents tested. Operating the foam column with 100.0 mg/L CTAB at 12 mL/min airflow rate is wherein the cellulase in the foamate is enriched more than 1 in both mass and activity, and it also yields higher mass and activity recovery (AR) than SDS at the same flow rates *(11)*.

As Pluronic F-68 does not provide an increase in cellulase enrichment, it means that the cellulase complex does not adsorb at the air-liquid interface, and only Pluronic F-68 adsorbs and forms a foam layer. The foam then carries out the Pluronic F-68 with the original liquid solution intact. It is unlikely that Pluronic F-68 forms a complex with cellulase because nonionic surfactants such as Pluronic F-68 usually do not interact with proteins *(22,23)*. Pluronic F-68 can be used to recover roughly 55% of the initial protein without the loss of enzymatic activity but with no protein enrichment in the foamate. As an SDS-assisted foam fractionation can concentrate cellulase *(11)*, it is likely that SDS forms a complex with cellulase because anionic surfactants such as SDS usually bond strongly with proteins *(22,23)*. Some cellulose components at pH 5.0, the pH used in foaming are on average cationically charged and will interact strongly with the anionic SDS *(22,23)*. When foam is not present (not aerated), SDS alone does not decrease the cellulase activity compared with the control

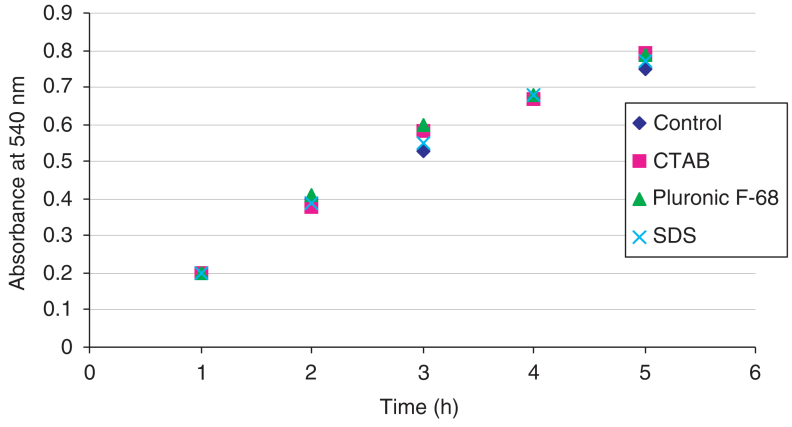

Fig. 4. Effect of adding different surfactants to a 200.0 mg/L cellulase solution without aeration. Absorbance response to the filter paper assay is shown in this figure.

run (of no added detergent) when tested in the filter-paper assay for 5 h. (as shown in Fig. 4). In that assay, a 200.0 mg/L cellulase solution is added to an equal value of a detergent (Pluronic F-68, SDS, or CTAB) to make 100.0 mg/L of detergent solution. Then the filter paper assay is performed, which results in an increased glucose concentration when there is an increased cellulose concentration. The increased glucose concentration reacts with the DNS solution causing a darkening of the solution. The darkened solution changes the absorbance readings determined by the plate reader and related back to the cellulose concentration. The activity is checked every hour for 5 h. The ER can be determined from the absorbance readings and is defined as the activity per unit volume of the foamate divided by the initial activity per unit volume of the solution before foaming *(11)*. The AE is defined as the activity per unit volume of the foamate divided by the initial activity per unit volume of the solution before foaming *(11)*. The MR is defined as the amount of protein in the foamate divided by the initial amount of protein *(11)*. The AR is defined as the total activity in the foamate divided by the total initial activity *(11)*.

$$\mathrm{ER} = \frac{C_{\mathrm{foam}}}{C_{\mathrm{initial}}} \tag{1}$$

$$\mathrm{AE} = \frac{A_{\mathrm{foam}}}{A_{\mathrm{initial}}} \tag{2}$$

$$\mathrm{MR} = \frac{C_{\mathrm{foam}} \times V_{\mathrm{foam}}}{C_{\mathrm{initial}} \times V_{\mathrm{initial}}} \tag{3}$$

$$\mathrm{AR} = \frac{A_{\mathrm{foam}} \times V_{\mathrm{foam}}}{A_{\mathrm{initial}} \times V_{\mathrm{initial}}} = \eta \times \mathrm{MR} \tag{4}$$

Thus, the damage occurring in the foam fractionation process of cellulase is probably caused by the foaming process, not from the surfactant added. The reorientation of a protein at a gas-liquid interface can cause surface denaturation, and is the main cause of protein denaturation in the foaming process *(30,31)*. Thus, this change in cellulase structure can result in a loss of enzymatic activity. SDS helps enrich the cellulase concentration as much as three and half times, but the activity only increases by 1.3-times at its maximum *(11)*. However, the addition of SDS only leads to a recovery of around 0.23 of the initial mass and only 0.1 of the initial activity. The addition of β-cyclodextrin with SDS creates a more dilute solution but does not provide any gain in the AE. The SDS and β-cyclodextrin pairing does not renature the enzyme well, in contrast to what has previously been reported in the literature *(32)*. CTAB tests are then carried out because it appears that CTAB and β-cyclodextrin work well with many of the proteins studied in the literature *(33–35)*. CTAB concentrates cellulase as well as SDS, but CTAB yields higher MR results. After foaming, cellulase specific activity decreases below that of the initial solution, which results in a difference between mass ER and AE ratio. Adding β-cyclodextrin (the stripping agent) solution increases the activity of the foamate as it dilutes the solution. The addition of artificial chaperones, which are substances to help proteins regain their desired folding (consisted of a detergent plus stripping agent) to the foam process, allows the cellulase to refold to its native state; thus, leading to an enzymatic activity increase. In this two step process, the detergent is likely to form a complex with the cellulase protein in the first step, preventing both aggregation and renaturation. In the second step, the stripping agent (cyclodextrin) takes away the detergent from the protein–detergent complex allowing much of the now concentrated protein to fold back to its native state.

This postulated mechanism is analogous to the well-studied natural chaperone system of GroEL/GroES *(33,34)*. In that system, GroEL first captures a denatured protein by binding to the exposed hydrophobic surfaces of that protein. Then, GroES interacts with GroEL to release the now natured protein. In the modified (for renaturation) foam fractionation process for cellulase, pictured here, the additional detergent (e.g., CTAB) binds with cellulase. CTAB does not necessarily unfold the native enzyme at room temperature, but it does dissolve any aggregated proteins *(33)*. Cationic detergents such as CTAB generally have a strong potential to bind with proteins. The surface tension curves (Fig. 5), shown here for cellulase, indicate that mixing CTAB with cellulase solution to form CTAB–cellulase complex makes the solution more surface active at least at a CTAB concentration of 0.5 m*M* (by lowering the surface tension) than CTAB or cellulase alone.

The resulting lower surface tension of the CTAB in a 0.2 mg/mL (200.0 mg/L) cellulase solution at a CTAB concentration before the CMC (1 m*M*) of CTAB likely indicates that there is a small interaction between cellulase and CTAB, particularly of the 0.5 m*M* concentration wherein the response

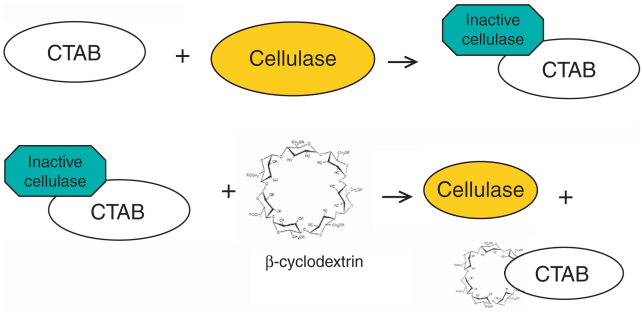

Fig. 5. Surface tension responses to an increase in CTAB or cellulase concentration in water and an increase in CTAB concentration in a 200.0 mg/L cellulase solution.

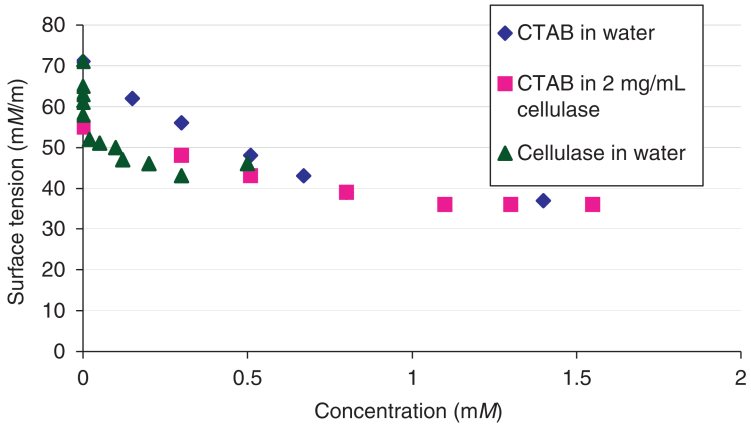

Fig. 6. Postulated mechanism for cellulase concentration in two steps. In step 1 cellulase and CTAB form a complex in order to concentrate the cellulase by foaming. In step 2 the CTAB is stripped away by the β-cyclodextrin.

is more than CTAB and cellulase alone. In order for the foam fractionation process to be able to concentrate a protein (such as cellulase) solution in the foamate, it is crucial that protein adsorption occurs on the foamed bubbles.

There are three possible mechanisms for adsorption of proteins in the presence of an interface and surfactants, as have been suggested *(36,37)*:

1. The protein and the surfactant do not form a complex but compete for adsorption sites on the bubble surface.
2. The protein and the surfactant form a complex, following the adsorption of only one complex at the interface.
3. Both protein and surfactant adsorb at the interface and form a solution complex.

After adsorption, foaming, and subsequent foam collapse, the now modified activity drops as suggested in the "inactive cellulase" state in the mechanism modeled in Fig. 6. The decrease in cellulase activity may be because of changes in both the secondary and tertiary structures *(30,37,38)*.

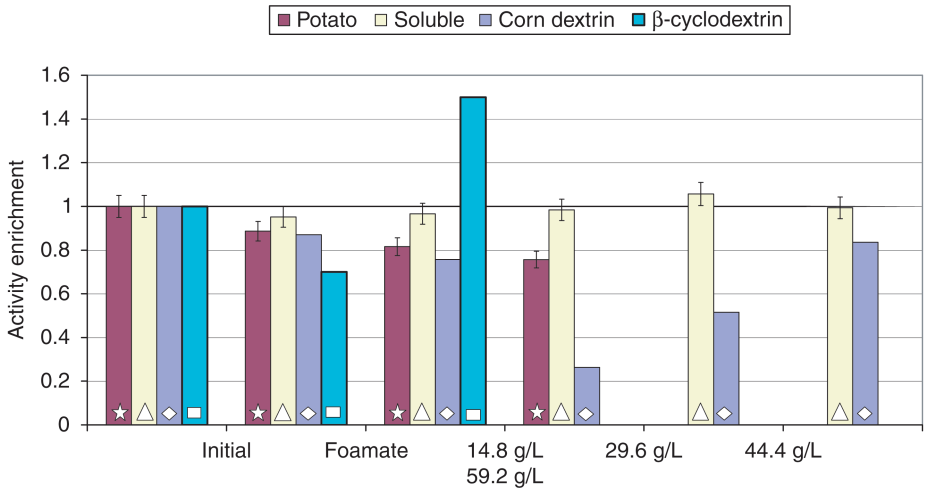

Fig. 7. Differences in the AE of cellulase before and after foam fractionationation (foamate state) refolded with β-cyclodextrin, potato starch, starch soluble, and corn dextrin at different concentration levels. The chart shows that β-cyclodextrin is the only effective detergent stripping agent at the 14.8 g/L level. The normalized AE of 1 corresponds to the initial activity of 0.1 unit/mL.

The presence of detergent in the foamate can perhaps also prevent the protein from refolding back into its desired native state, and thus, retain its proper secondary and tertiary structure. As the initial detergent concentration (100.0 mg/L CTAB = 0.27 m*M*) is below the CMC of 1 m*M*, the detergent–protein complex is likely not to be in a micelle form *(39)*.

Restoration of the cellulase activity is performed by adding β-cyclodextrin solution to complete the artificial chaperone process. Because cyclodextrin binds more strongly to the nonpolar segment of the detergent than it does to a protein, it has the ability to separate a detergent from a protein *(33)*. From thermodynamic measurements, it has been demonstarted that cyclodextrin binds strongly to the hydrophobic segment of a detergent *(40–43)*. Because cyclodextrin can strip away the detergent from a protein–detergent complex the protein is now free to refold to its desired native state. As previously mentioned straight chain dextrins and starches are unable to remove the detergents from cellulase even at significantly higher concentrations than the β-cyclodextrin levels typically used for refolding. Results for the refolding of the foamate cellulase are shown in Fig. 7 for potato starch, starch soluble, corn dextrin, and are compared with β-cyclodextrin up to the 14.8 g/L level. It is noted that the 26.6 g/L, 44.4 g/L, and 59.2 g/L tests were carried out with corn dextrin, thinking at first that high levels kept improving the activity of cellulase. It turned out that glucose impurities present in the corn dextrin give a false-positive when using the filter paper assay. Athough there is some refolding with corn dextrin (as described by the filter paper assay, with a correction for the contaminating glucose), it is still small compared with β-cyclodextrin refolding.

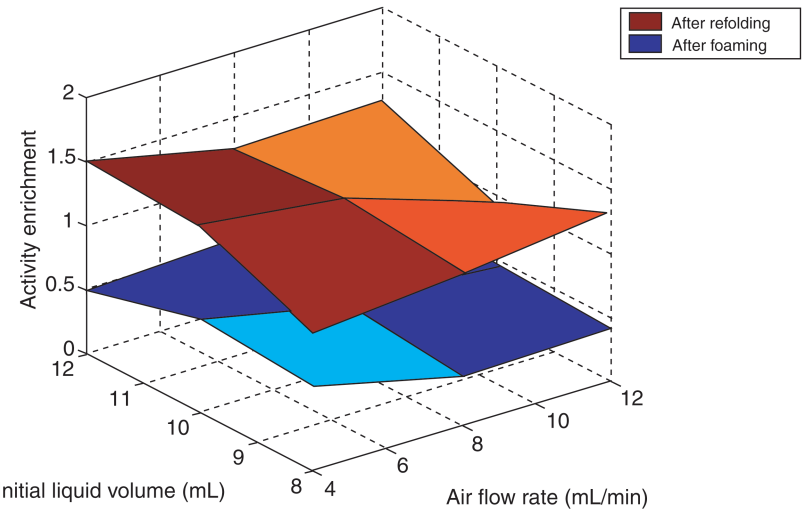

Fig. 8. Cellulase AE ratio for the foam fractionation of 200.0 mg/L Cellulase with 100.0 mg/L CTAB solution. The top graph illustrates the results from refolding with β-cyclodextrin solution.

However, the use of both CTAB and β-cyclodextrin together does not always refold an entire denatured protein *(32)*. This can be realized when the specific activity is calculated. The specific activity of cellulase occurs at its highest possible value before it is subjected to foam fractionation. After foam fractionation and refolding, the specific activity does not reach its initial value of 0.1 unit/mg indicating that all of the activity is not recovered and the entire denature molecule is not refolded *(11)*.

CTAB-assisted foam fractionation may concentrate different components of the cellulase complex than SDS-assisted foam fractionation. The operating pH is 5.0, but not all of pI's of the cellulase components are at 5.0. The most abundant component of cellulase produced from *Trichoderma reesei* is cellobiohydrolase I, which has a pI of 3.9 *(44,45)*. Cellobiohydrolase I likely has a net negative charge at pH 5.0 and interacts strongly with the cationic CTAB, but it may not interact as well with SDS because both are negatively charged. The cellulase component, β-glucosidase, has a pI around 6.5 *(29)*. It likely has a net positive charge at the operating pH of 5.0, so SDS probably binds stronger with this component than CTAB. However, the foam fractionation process does not have a high selectivity for distinguishing proteins with similar pI's *(7)*, so it is likely that other cellulase components are in the foamate as well. More experiments using two-dimensional gel electrophoresis or high-performance liquid chromatography are needed to determine if CTAB and SDS separate components of cellulase during the foam fractionation process. The results of applying the artificial chaperone system to the cellulase foam fractionation process are shown in previous study *(12)*. Typical results for the AE and the AR are shown in Figs. 8 and 9, respectively.

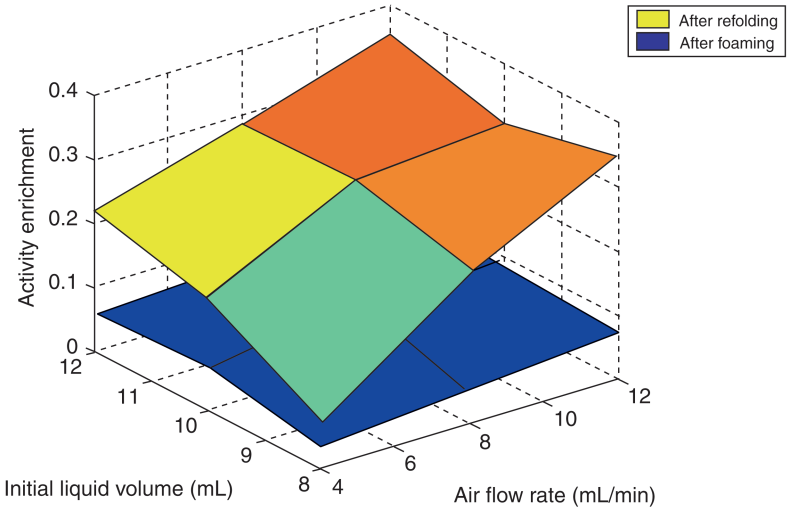

Fig. 9. Cellulase AR for the foam fractionation of 200.0 mg/L cellulase with 100.0 mg/L CTAB solution. The top graph illustrates the results from refolding with β-cyclodextrin solution.

The example underlying Figs. 8 and 9 uses a mixture of 200.0 mg/L cellulase and 100.0 mg/L CTAB as the initial solution, and the foamate is later diluted with β-cyclodextrin. There is a trade off between enrichment and recovery in this foam fractionation process. At the condition where the MR is the lowest, the mass ER is the highest. Therefore, it is difficult to determine the best operating conditions at one of these four points. Operating the column with a 12 mL/min airflow rate and a 12 mL initial volume and a 100.0 mg/L CTAB solution, it is chosen as the best point to operate (in the range of values tested) because it has the highest AR (*see* Fig. 9) compared with other points *(46–49)*. Just concentrating a denatured enzyme solution does not necessarily increase the value of the enzyme. What is needed is to increase the AE as well. It is noted that the discussed modified foam fractionation process may not be suitable for concentrating cellulase because of the loss in enzymatic activity.

Conclusions

The decrease in surface tension of a mixture of a detergent and a protien, compared with the detergent and protein alone, indicates the possibility of a detergent–protein complex. The complex formation is consistent with the hypothesis that the detergent–protein complex adsorbs on the bubble surface and rises out of the liquid phase into the foam phase. Electrostatic interactions play an importat role in SDS–lysozyme foam fractionation process because the SDS is anionic and lysozyme is positively charged at the tested pH. Moreover, neither CTAB (cationic) nor Pluronic F-68 (nonionic) by themselves can concentrate lysozyme (a positively charged enzyme) in the

foam fractionation process. It is recommended that future research into this should include modeling the early time data during the refolding of cellulase with β-cyclodextrin to elucidate the early time dynamics in much the same manner as previously observed for the similar biomolecular mechanism of a substrate and enzyme in an enzyme kinetic reaction scheme.

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