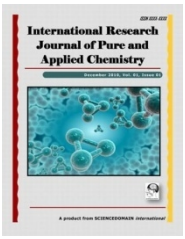

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Effect of N-Alkyl Trimethylammonium Bromide (C_nTAB) Cationic Surfactant Hydrophobicity on Cellulase Intrinsic Fluorescence

Derek D. Kim[1] and Ruben M. Savizky[1*]

[1]Department of Chemistry, The Cooper Union, 41 Cooper Square, New York NY 10003, USA.

Authors' contributions

This work was carried out in collaboration between all authors. Author DDK designed the study, performed the analyses, and wrote the first draft of the manuscript. Author RMS managed the analyses of the study and supervised the work. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To determine the effect of the hydrophobic forces involved in the interaction of cellulase (endo-β-1,4-glucanase from *Aspergillus niger*), and cationic surfactant n-alkyl trimethyl ammonium bromides (C_nTAB), with varying chain lengths (n = 10, 12, 14, 16, 18), steady-state fluorescence spectroscopy using tryptophan as a probe was employed.
Study Design: Using intrinsic tryptophan emission, a Stern-Volmer analysis of steady-state fluorescence spectroscopy data was used.
Place and Duration of Study: Department of Chemistry, The Cooper Union between January 2009 and April 2010.
Methodology: Intrinsic cellulose fluorescence was quenched by the addition of cationic surfactants. This quenching, which might occur by dynamic or static collisional mechanisms, was measured by monitoring the decrease in fluorescence intensity as a function of the surfactant concentration. A Stern-Volmer plot was then generated to obtain values for K_{SV}, the Stern-Volmer constant for the interaction.
Results: No appreciable quenching occurred for the lower chain C_nTABs ($C_{10}TAB$ and $C_{12}TAB$). A linear form of the Stern-Volmer equation modeled the behavior of cellulase

**Corresponding author: Email: rsavizky@cooper.edu;*

quenching by C_{14}TAB, C_{16}TAB, C_{18}TAB, and the K_{SV} values were determined to be 5.9 x 10^{-5} μM^{-1} (59 M^{-1}), 1.1 x 10^{-4} μM^{-1} (110 M^{-1}), and 1.4 x 10^{-4} μM^{-1} (140 M^{-1}), respectively, with R^2 values greater than 0.90 but less than 0.95. The data for the experiments involving C_{12}TAB and C_{14}TAB disagreed significantly with earlier published work in that they differed significantly in the extent of quenching and emission wavelength shift observed from cellulase fluorescence upon addition of the surfactants.

Conclusion: None of the results obtained in this experiment were able to confirm the biphasic behavior of endoglucanase. The K_{SV} values obtained from the results are sufficient to claim that higher chain surfactants are more effective quenchers of cellulase fluorescence and consequently, that the hydrophobic forces play a great role in cellulase-C_nTAB interactions. A modified form of the equation (having a second order with respect to the concentration of the quencher) distinguishing and modeling both dynamic and static quenching can possibly be applied in the future studies to improve the accuracy of the Stern-Volmer model. Although this would enable to distinguish and perhaps more accurately model the two types of quenching exhibited by the same fluorophore, it would require additional experiments such as measuring fluorescence lifetimes (τ) and/or testing for the effect of temperature.

Keywords: Cellulose; cationic surfactants; fluorescence; Stern-Volmer analysis.

1. INTRODUCTION

The fungus *Aspergillus niger* is an important commercial source of industrial cellulase, not only in the food and textile industries but also in pharmaceutical industries, and endo-β-1,4-glucanase is the main component for cellulose degradation by *Aspergillus niger* [1]. Hence, cellulase (endo-β-1,4-glucanase) from *Aspergillus niger* was chosen to be studied in this work.

Endo-β-1,4-glucanase from *Aspergillus niger* (abbreviated EglA) consists of a 'jelly-roll' fold with two antiparallel β-sheets (outer A and inner B). β-Sheet A contains six strands (A1-A6), and β-sheet B contains nine strands (B1-B9). In terms of polarity, the hydrophilic face of β-sheet A is exposed to solvent, while the hydrophilic face of β-sheet B forms a long open cleft which is the binding site for substrate [1]. In the native state structure, β-strand A has five tryptophan (Trp 45, 49, 51, 85, 218) residues, β-strand B has three (Trp 120, 144, 147) and Trp 22 is invariant [2].

Access of cellulase to the cellulose network is crucial for a successful hydrolysis, and this can be controlled by the quality of pretreatment. While one important factor contributing to the complex nature of cellulose hydrolysis is the presence of lignin, pretreatment is believed to influence the extent of cellulose hydrolysis in the absence of lignin as well. Other parameters that can determine the rate and/or the effectiveness of hydrolysis are the degree of crystallinity of cellulose and whether or not the ratio of cellulase and β-glucosidase is optimized, since inhibition of cellulase by cellobiose causes inefficient hydrolysis [3]. Finally, and perhaps most interestingly, there have been findings that adding surfactants can enhance the enzymatic cellulose hydrolysis.

Enhancement of enzymatic cellulose hydrolysis by the addition of surfactants has been studied and reported by several authors in the literature. Some of these authors have agreed that its effect has to do with adsorption of enzyme, although the details of their claims do not exactly coincide. In early work, Ooshima et al. found that the adsorption of the cationic

surfactant Q-86W on cellulose obeys a Langmuir isotherm (which relates adsorption of molecules on a solid surface to concentration of a medium above the solid surface at a fixed temperature), suggesting that surfactants adsorb to the cellulose during hydrolysis [4]. Kim et al. had a different explanation, proposing that surfactants adsorb at the air-liquid interface [5]. Consequently, the degree of enzyme deactivation (denaturation) and loss of enzyme adsorption during agitation in the hydrolysis would be inhibited. With the addition of sufficient surfactants, the cellulase can be stabilized and its use can be prolonged.

Others have taken a kinetics analysis approach to explain the phenomenon of the enhancement of enzymatic cellulose hydrolysis by adding surfactants. From their kinetic studies, Kaar and Holtzapple have shown that surfactants such as Tween 20 (polyoxyethylene sorbitan monolaurate) and Tween 80 (polyoxyethylene sorbitan monooleate) can make active sites more available; this would improve the enzymatic absorption constants and then lead to an increase in the effective hydrolysis rate compared to hydrolysis without the surfactant (as evidenced by the conversion of cellulose being increased by 42%) [6]. They also observed that Tween prevented thermal deactivation of the enzymes, which allows for the kinetic advantage of higher temperature hydrolysis. The conclusion was that the surfactant improves cellulose hydrolysis through three effects: enzyme stabilization, lignocellulose disruption, and higher conversion rate.

Another group of researchers, Eriksson et al. has shown through their activity and fluorescence experiments that improved conversion of lignocellulose by adding anionic and non-ionic surfactants can be attributed to the increased cellulase stability in their presence, and hence the reduction of enzyme denaturation during the hydrolysis [7]. More specifically, they concluded that upon the addition of surfactants, the unproductive enzyme adsorption to the lignin part of the substrate is reduced, due to the hydrophobic interaction of surfactant with lignin on the lignocellulose surface, which releases unspecifically bound enzyme.

Sodium carboxymethyl cellulose (abbreviated CMC) is a substituted polymeric cellulose-derived substrate analog that is water soluble. In near neutral to weak basic solution, sodium carboxymethyl cellulose exists as a macro anion with multi-charges (i.e. anionic polyelectrolyte) because of the dissociation of the carboxymethyl group (-CH_2-COOH), while its disaccharide unit has strong hydrophobicity [8]. Polyelectrolytes have a tendency to form micellar aggregates or micelle-like clusters with surfactants of opposite charge. For CMC, an anionic polyelectrolyte, the driving force of the aggregation (cooperative) of cationic surfactants has been found to be of electrostatic and hydrophobic origin; that is, the association was strengthened as the charge density of the polysaccharide or the hydrophobic chain length of the surfactant increased [9]. Chakraborty et al. have employed a variety of thermodynamic measurements to further characterize the interaction between CMC and CTAB surfactants [10]. Using tensiometry, conductometry, isothermal titration microcalorimetry, and turbidity measurements, they were able to conclude that the interaction between these species is driven by a combination of enthalpic and entropic contributions, due to both hydrophobic and electrostatic effects.

2. MATERIALS AND METHODS

De-ionized water was provided by the Cooper Union chemistry department. Sodium acetate buffer solution, 3 M (Molecular Biology Tested), pH 5.2±0.1 (25ºC), cellulase from *Aspergillus niger* (Cellulaza*1,4-(1,3:1,3)-β-D-Glucan 4-glucanohydrolase, EC 3.2.1.4, molecular weight 31000 g/mol, with an activity of 0.8 units/mg), carboxymethyl cellulose (sodium salt), decyltrimethylammonium bromide (C_{10}TAB), dodecyltrimethylammonium

bromide (C_{12}TAB), Tetradecyltrimethylammonium bromide (C_{14}TAB), Hexadecyltrimethylammonium bromide (C_{16}TAB), and Octadecyltrimethylammonium bromide (C_{18}TAB) were purchased from Sigma-Aldrich Co. (St. Louis, MO, United States).

Fluorescence measurements were done using Varian Cary Eclipse fluorescence spectrophotomer with a Peltier thermostatted multicell holder accessory that had an electromagnetic stir control and a temperature controller with a range of –10ºC to +100ºC. A quartz fluorimeter cell (cuvette) with a 3.5 mL maximum volume capacity was purchased from McCarthy Scientific Co. (Fallbrook, CA) and was used throughout all fluorescence measurements. The SCAN mode was used to take each fluorescent measurement with respect to a range of wavelengths. The excitation wavelength was set at 280 nm and the emission was recorded from 290 nm to 410 nm in order to trace the tryptophan fluorescence. The resulting slit widths were 2.5 nm and 5 nm for excitation and emission, respectively (which are close to Rastegari *et al.*'s setting of 3 nm and 5 nm [2]), and PMT voltage was selected to be 850V. The Peltier block temperature was set as 25ºC to run the experiment at room temperature (following Rastegari *et al.*'s setting) and the smoothing option was selected to smooth out the spectra for better viewing. The scanning speed was set as slow (120 nm/min) to obtain a good resolution of the spectra, and since the emission wavelength range was 120 nm (from 290 nm to 410 nm), each scan took approximately 1 minute.

Laboratory procedures for preparing the necessary solutions for the fluorescence quenching experiments were based on the method by Rastegari et al. [2] with some modifications. Three different solution components were added the cuvette: buffer, cellulase/(sodium) carboxymethyl cellulose (abbreviated CM-cellulose from here on) solution, and cationic surfactant (C_nTAB) solution. 50 mM sodium acetate buffer was prepared by diluting the 3 M sodium acetate buffer solution (pH 5.2±0.1) with de-ionized water. This pH is slightly more basic than the isoelectric pH of cellulase and that of CM-cellulose (3.67, 4.6, respectively) to ensure that they both maintain a negative charge density [2,9,11]. A batch of 900 mL was prepared at a time (hence 15 mL 3 M sodium acetate buffer with 885 mL de-ionized water), and it was used for all solutions.

The sample solution was to consist of cellulase (enzyme) and CM-cellulose dissolved in 50 mM sodium acetate buffer. The concentration of the cellulase in the sample solution to be used in the cuvette for the fluorescence measurement was determined from the optical density of appropriate solutions using the theoretical extinction (molar absorption) coefficient at 280 nm of 97,010 $M^{-1}cm^{-1}$ [12]. Using Beer's Law with l = 1 cm for the optical path length for cuvette and a value of 0.48505 for absorbance, the concentration of cellulase in the cuvette for measuring fluorescence was conveniently chosen to be 5.0 µM. The concentration of CM-cellulose in the sample solution used for the fluorescence measurement was 0.5% (w/v), based on A.A. Rastegari et al.'s method [2].

For each fluorescence measurement, 3.0 mL was set as the total volume of solution in the cuvette. Of the 3.0 mL, the cellulase/CM-cellulose stock solution was only half filled (1.5 mL) each time to allow the addition of other two solution components, buffer and C_nTAB stock solution. Thus, to achieve the necessary concentration of 5.0 µM of the cellulase in the sample solution in cuvette, the stock solution (100 mL total volume) of cellulase/CM-cellulose was freshly prepared before each experiment with double the aforementioned concentrations.
Each of the five cationic surfactant (C_nTAB) stock solutions (100 mL total volume) was prepared by dissolving 7.5 mM of surfactant in 50 mM sodium acetate buffer prepared previously. Low heat was applied when necessary.

After the appropriate settings were applied to the fluorescence (as described above), 3.0 mL of 50 mM sodium acetate buffer was pipetted into a fluorimeter cuvette, and the cuvette was inserted in one of the cells in the Peltier block. The fluorescence reading was zeroed, and the quenching experiment was initiated. A total of 16 fluorescence readings were taken for each quenching experiment. The concentration of the surfactants was varied from 0 μM to 3000 μM (i.e. mole ratio of C_nTAB to cellulase ranges from 0 to 600), while the concentration of the cellulase remained as 5.0 μM (1.5 mL of cellulase/CM-cellulose standard solution containing 10 μM cellulase was added each time for all readings). The amount of buffer added was adjusted accordingly to fill the remaining portion of the set total volume of 3.0 mL. None of the solutions appeared visibly turbid or seemed to contain a precipitate.

All three solution contents in the cuvette were stirred for 5 minutes (at 600 rpm) prior to recording the fluorescence spectrum by using the internal stir control of the Peltier block and a stir bar specifically designed for spectroscopy cuvettes (purchased from SigmaAldrich Co., Catalogue# Z363545). Between every measurement, the cuvette was thoroughly washed out with de-ionized water and a cuvette cleaner concentrate (purchased from SigmaAldrich Co., Catalogue# 61257).

3. RESULTS AND DISCUSSION

CM-cellulose is a polyelectrolyte and therefore, it forms micellar aggregates or micelle-like clusters with surfactants of opposite charge. The driving force of the aggregation (cooperative) of cationic surfactants with CM-cellulose has previously been found to be of electrostatic and hydrophobic origin; that is, the association was strengthened as the charge density of the polysaccharide or the hydrophobic chain length of the surfactant increased [9]. To determine the effect of the latter source during the surfactant's interaction with cellulase/CM-cellulose, the steady-state intrinsic fluorescence data (using tryptophan as a probe) of cellulase were obtained after adding various concentrations of the cationic surfactants C_nTAB of different hydrophobic chain lengths (n = 10, 12, 14, 16, 18), in a procedure similar with those described in the literature on similar systems [11,13,14]. If quenching of cellulase fluorescence were to occur, this would be an indication that the tryptophan residues of cellulase are less exposed to the solvent upon the binding of C_nTAB ions.

3.1 Quenching of Intrinsic Cellulase Fluorescence by N-Alkyltrimethylammonium Bromides (C_nTABs)

Figs. 1-5 show the selected fluorescence intensity measurements acquired in the emission wavelength range 290 nm – 410 nm after being excited at 280 nm for varying concentrations of C_{10}TAB, C_{12}TAB, C_{14}TAB, C_{16}TAB and C_{18}TAB (0 – 3000 μM). It can be seen that for the shorter chain-length surfactants (n=10 or 12), there was no significant change in cellulose fluorescence. However, as the length of the carbon chain increased, the intrinsic fluorescence was quenched in a concentration dependent manner.

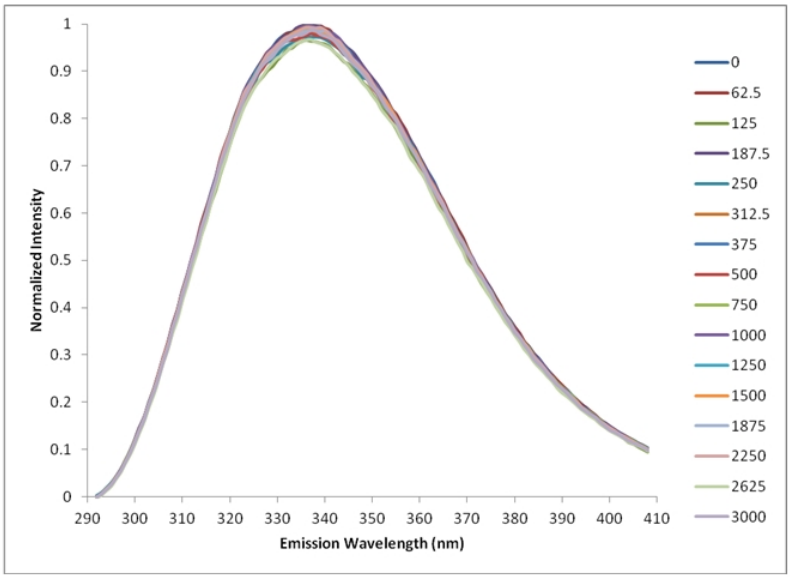

Fig. 1. Fluorescence emission spectra of cellulase from a solution of 5.0 µM cellulase, 0.5% (w/v) CM-Cellulose as a function of C_{10}TAB concentration (ranging from 0-3000 µM), all in 50 mM sodium acetate buffer pH 5.0, and at 25ºC ($\lambda_{excitation}$ = 280 nm). The fluorescence intensity was normalized by dividing all spectra by the maximum intensity at λ_{max} (337 nm) in the absence of C_{10}TAB, which had a value of 614 a.u

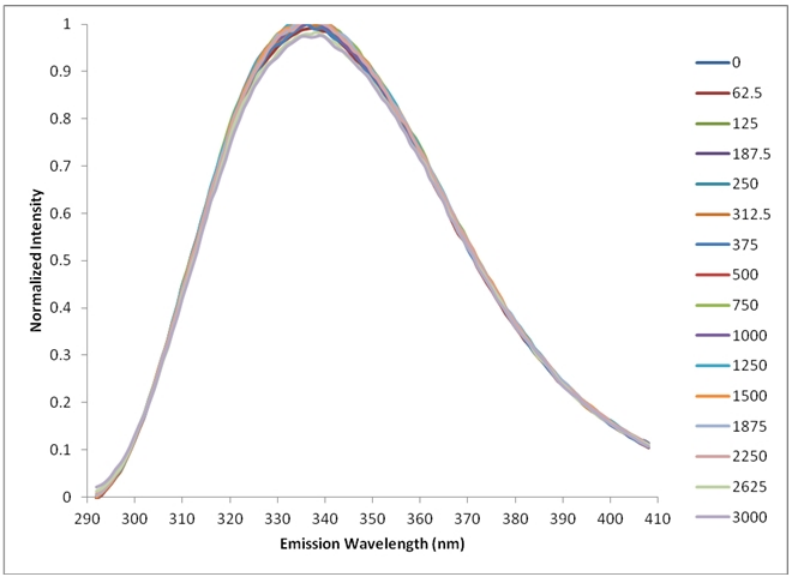

Fig. 2. Fluorescence emission spectra of cellulase from a solution of 5.0 µM cellulase, 0.5% (w/v) CM-Cellulose as a function of C_{12}TAB concentration (ranging from 0-3000 µM), all in 50 mM sodium acetate buffer pH 5.0, and at 25ºC ($\lambda_{excitation}$ = 280 nm). The fluorescence intensity was normalized by dividing all spectra by the maximum intensity at λ_{max} (337 nm) in the absence of C_{12}TAB, which had a value of 551 a.u

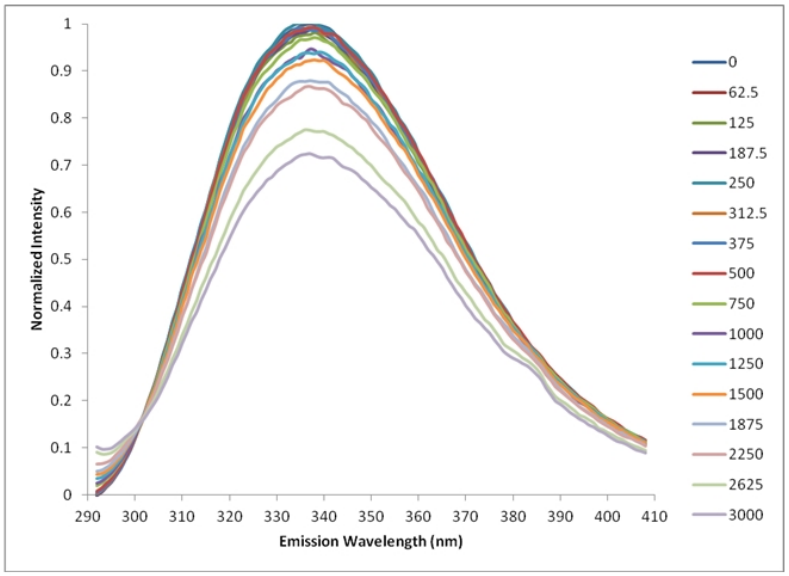

Fig. 3. Fluorescence emission spectra of cellulase from a solution of 5.0 µM cellulase, 0.5% (w/v) CM-Cellulose as a function of C_{14}TAB concentration (ranging from 0-3000 µM), all in 50 mM sodium acetate buffer pH 5.0, and at 25ºC ($\lambda_{excitation}$ = 280 nm). The fluorescence intensity was normalized by dividing all spectra by the maximum intensity at λ_{max} (337 nm) in the absence of C_{14}TAB, which had a value of 576 a.u

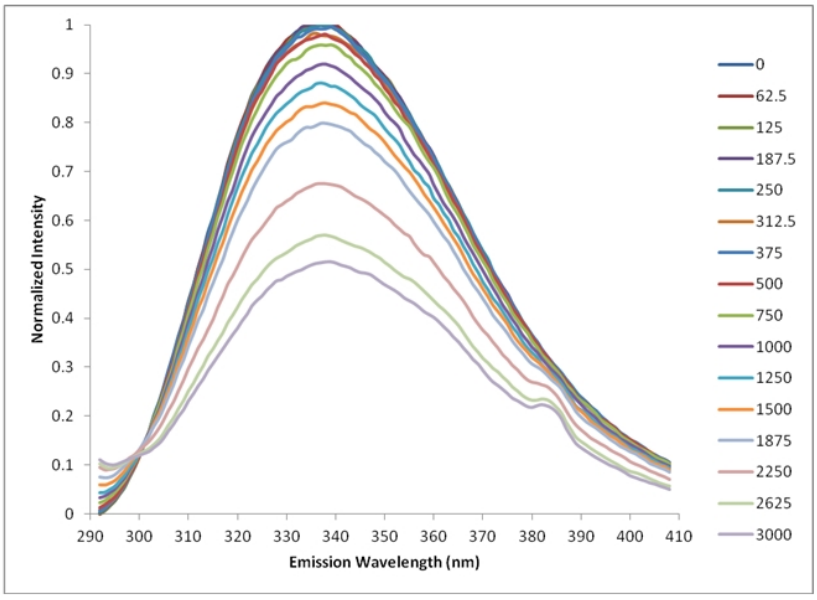

Fig. 4. Fluorescence emission spectra of cellulase from a solution of 5.0 µM cellulase, 0.5% (w/v) CM-Cellulose as a function of C_{16}TAB concentration (ranging from 0-3000 µM), all in 50 mM sodium acetate buffer pH 5.0, and at 25ºC ($\lambda_{excitation}$ = 280 nm). The fluorescence intensity was normalized by dividing all spectra by the maximum intensity at λ_{max} (337 nm) in the absence of C_{16}TAB, which had a value of 623 a.u

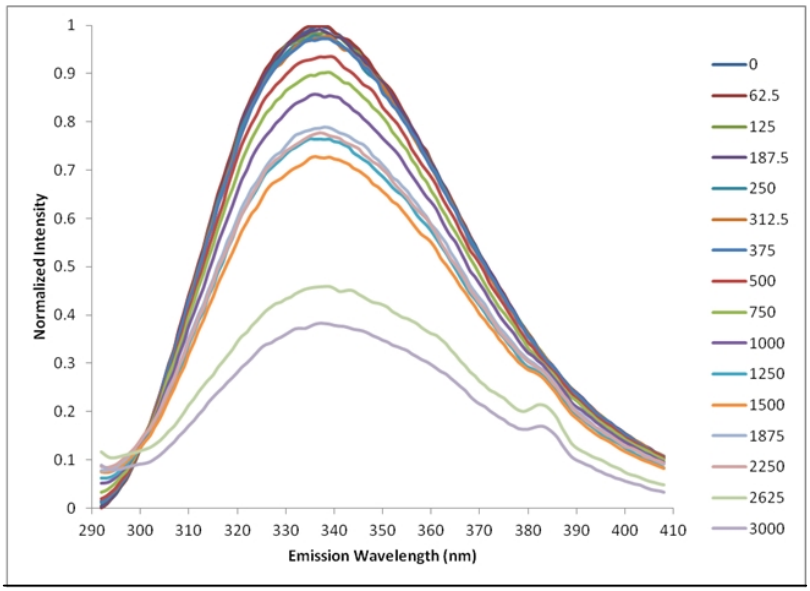

Fig. 5. Fluorescence emission spectra of cellulase from a solution of 5.0 μM cellulase, 0.5% (w/v) CM-Cellulose as a function of C_{18}TAB concentration (ranging from 0-3000 μM), all in 50 mM sodium acetate buffer pH 5.0, and at 25 °C ($\lambda_{excitation}$ = 280 nm). The fluorescence intensity was normalized by dividing all spectra by the maximum intensity at λ_{max} (337 nm) in the absence of C_{18}TAB, which had a value of 623 a.u

3.2 Stern-Volmer Plots of Cellulase in Varying Concentrations of Cationic Surfactant n-Alkyl Trimethylammonium Bromide (C_nTAB)

Dynamic (collisional) fluorescence quenching is described by the Stern-Volmer equation (1)

$$\frac{F_0}{F} = 1 + k_q \tau_0 [Q] = 1 + K_{SV}[\mathrm{Q}] = \frac{\tau_0}{\tau} \quad (1)$$

where F_0 and F are the fluorescence intensities with the given excitation wavelength (in the case of cellulase, it is excitation wavelength for the tryptophan residue, which is supposed to be around 280 nm) in the absence and presence of quencher, respectively, k_q is the bimolecular quenching constant (reflecting the efficiency of quenching or the accessibility of the fluorophores to the quencher), τ_0 and τ are the lifetimes of the fluorophore in the absence and presence of quencher, respectively, and [Q] is the concentration of quencher (the surfactant). The Stern-Volmer quenching constant, K_{SV} is defined as the product of k_q and τ_0. If the quenching is known to be dynamic, the Stern-Volmer constant will be represented by K_D.

It is important to note that the fluorescence intensities for all the points (with varying surfactant concentrations) are taken at the wavelength at which maximum fluorescence intensity occurred with absence of surfactant (i.e. just the cellulase/CM-cellulose solution and buffer) for each trial. From the plots, values of K_{SV} were determined by fitting a linear regression to the number of data points that gave a reasonable R^2 value (≥ 0.90). In general, a linear Stern-Volmer plot means that there is only a single population of fluorophores that is all equally accessible to the quencher [15-19]. If two fluorophore classes are present, and

one of them is not accessible to the quencher, then the Stern-Volmer plots deviate from linearity toward the *x*-axis. Such a phenomenon can sometimes be observed for quenching of tryptophan fluorescence in proteins by polar or charged quenchers [15-19]. Because these polar or charged quenchers do not easily penetrate the hydrophobic interior of proteins, only the tryptophan residues on the surface of the protein are quenched.

Fig. 6 shows the Stern-Volmer plots for C_{14}TAB, C_{16}TAB and C_{18}TAB. It can be seen that a linear relationship is observed within a large concentration range for these surfactants (as evidenced by the square of the correlation coefficient, R^2), although the range decreases with chain length. From this analysis, Stern-Volmer constants (K_{SV}) values of $5.79*10^{-5}$ μM^{-1}, $1.07*10^{-4}$ μM^{-1} and $1.42*10^{-4}$ μM^{-1} can be calculated for C_{14}TAB, C_{16}TAB and C_{18}TAB, respectively. The results sustain the fact that these cationic surfactants bind to the negative charges of cellulase and CM-cellulose polymer monomers, and that hydrophobic interactions do play a significant role.

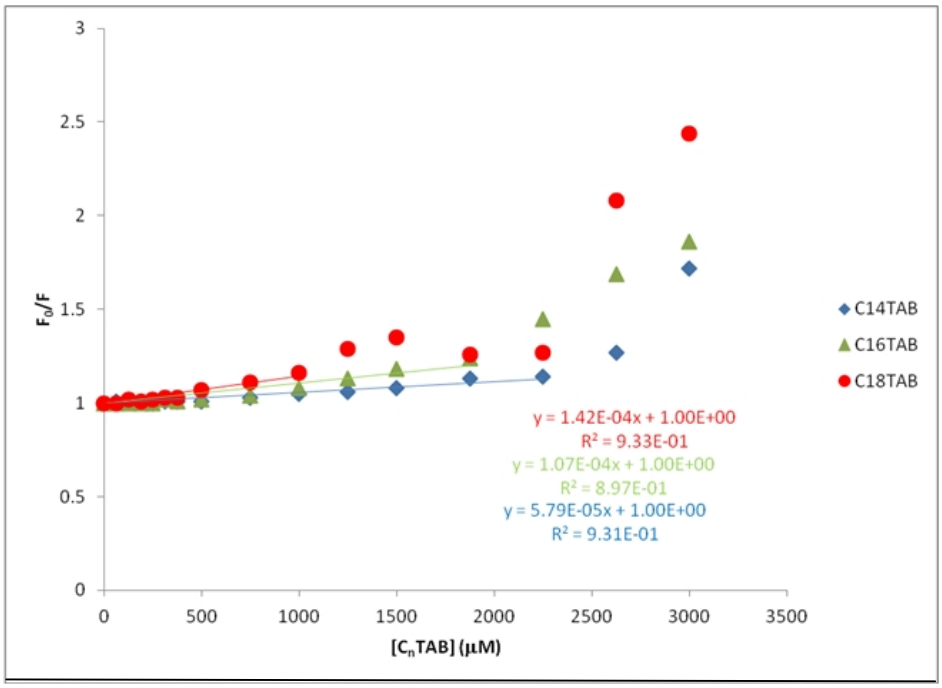

Fig. 6. Generation of Stern-Volmer plots for various C_nTAB surfactants

The Stern-Volmer equation for the cellulase fluorescence quenching by C_nTAB was only applicable to some extent. The three Stern-Volmer plots generated from these experiments for C_{14}TAB, C_{16}TAB, and C_{18}TAB show slope values (equivalent to the Stern-Volmer constant, K_{SV}) values of 5.79×10^{-5} μM^{-1} (57.9 M^{-1}), 1.07×10^{-4} μM^{-1} (107 M^{-1}), and 1.42×10^{-4} μM^{-1} (142 M^{-1}), respectively, and R^2 values greater than 0.90 but less than 0.95. While this may be sufficient to declare that hydrophobic effects play a great role in cellulase quenching by the higher chain C_nTABs, it may not be enough to confidently declare that quenching of cellulase fluorescence by C_nTAB (at the tested concentrations) follows a linear form of Stern-Volmer model (one type of quenching, referred to as parallel quenching). Deviations of Stern-Volmer can be due to the simultaneous presence of dynamic (by collision) and static quenching (complex formation in the ground state) with the same quencher [13]. A positive

deviation (upward curvature and concave towards the intensity axis) from the *x*-axis usually indicates presence of both static and dynamic quenching [15-19]. Then, the Stern-Volmer equation can be modified as

$$\frac{F_0}{F} = \left(1 + K_{dynamic}[Q]\right)(1 + K_{static}[Q]) = 1 + \left(K_{dynamic} + K_{static}\right)[Q] + K_{dynamic}K_{static}[Q]^2 \quad (2)$$

so that it is second order with respect to [Q] to account for the upward curvature observed when both static and dynamic quenching occur for the same fluorophore. However, this would require additional experiments such as measuring fluorescence lifetimes and/or testing for the effect of temperature [15-19].

Despite the simplicity and the usefulness of the Stern-Volmer equation in helping to describe collisional fluorescence quenching, there is a significant downside: the result of a linear Stern-Volmer plot does not absolutely prove that collisional quenching of fluorescence has occurred. An intriguing fact is that static quenching also follows the Stern-Volmer equation and generates linear Stern-Volmer plots [15,16]. As described above, dynamic (collisional) quenching, which can be described by the Stern-Volmer equation, results from diffusive encounters between the fluorophore and quencher during the lifetime of the excited state, which indicates that the process is time-dependent. Quenching can also happen from the formation of a nonfluorescent complex between the fluorophore and quencher, which previously was defined as static quenching. When this fluorophore/quencher complex absorbs light, it immediately returns to the ground state without emission of a photon [11,12]. The most accurate way to distinguish dynamic and static quenching is the measurement of fluorescence lifetimes, although these can be difficult to obtain. Besides measurement of fluorescence lifetimes, dynamic and static quenching can be set apart by their contrasting dependence on temperature. Since dynamic quenching depends upon diffusion, higher temperatures will cause more collisions among molecules to occur and thus faster diffusion and larger extent of collisional quenching (higher value of biomolecular quenching constant (k_q), which also means a greater value of K_{SV}. For the case of static quenching on the other hand, increased temperature will generally result in the dissociation of weakly bound complexes and decrease of their stability, leading to smaller extent of static quenching (lower value of the static quenching constant (K_S)) [15,16].

4. CONCLUSION

To determine the effect of the hydrophobic forces involved in the cationic surfactant n-alkyl trimethylammonium bromide (C_nTAB)'s interaction with cellulase/CM-cellulose, the steady-state intrinsic fluorescence data (using tryptophan as a probe) of cellulase were obtained after adding various concentrations of the cationic surfactants C_nTAB of different hydrophobic chain lengths (n = 10, 12, 14, 16, 18). No noticeable quenching occurred for the lower chain C_nTABs (C_{10}TAB and C_{12}TAB), while a clear increasing trend in K_{SV} values were obtained for C_{14}TAB, C_{16}TAB, and C_{18}TAB were 5.79 x 10^{-5} μM^{-1}, 1.07 x 10^{-4} μM^{-1} and 1.42 x 10^{-4} μM^{-1}, respectively, with R^2 values all greater than 0.90 but less than 0.95.

The results for the experiments involving C_{12}TAB and C_{14}TAB were not reproducible from the work of Rastegari et al. [2] in that they differed significantly in the extent of quenching and emission wavelength shift upon addition of the surfactants observed from fluorescence spectra. Specifically, the literature reports relatively constant fluorescence intensities of approximately 200-210 a.u. at a maximum wavelength of 372 nm in the presence of C_{12}TAB, and a much different range of approximately 350-750 a.u. at a maximum wavelength of 362

nm in the presence of C_{14}TAB (with an *increase* in intensity due to an increase in surfactant concentration). Our findings show the maximum wavelength around 337 nm independent of the surfactant, with intensities that were relatively constant in the presence of C12TAB and *decreased* significantly when C_{14}TAB was added. The differences cannot be attributed to differing properties of the cellulose and/or carboxymethyl cellulose, as they had identical molar masses and degrees of substitution. In addition, while Rastegari et al. observed significant changes in slope in the quenching plots, none of the surfactants tested (even the additional three surfactants with higher chain lengths, i.e. C_{14}TAB, C_{16}TAB, and C_{18}TAB) indicated this behavior, which made it unable to confirm Rastegari et al.'s claim that endoglucanase exhibits a biphasic behavior.

To improve the accuracy of the Stern-Volmer model, a modified form of equation (2) distinguishing and modeling both dynamic and static quenching can be used. Although this would be able to distinguish and perhaps more accurately model the two types of quenching exhibited by the same fluorophore, it would require additional experiments involving fluorescence lifetimes (τ) and/or effect of temperature [15-19].

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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