Study of interaction between *Aspergillus niger* cellulase (ANC) and Cetyltrimethylammonium Bromide (CTAB) using surfactant membrane selective electrode

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Abstract

The interaction of cetyltrimethylammonium bromide, CTAB, to *Aspergillus niger* cellulase, ANC, has been studied using potentiometric technique. Binding isotherms were obtained from the potentiometric technique and using the Wyman binding potential model, apparent binding constants were calculated. The obtained binding isotherms have been analysed and interpreted using the Wyman binding potential. The Gibbs free energy change calculated on the basis of the Wyman binding potential concept decreases with increasing amount of binding. The affinity of binding increased with increasing temperature, indicating an endothermic and essentially entropy driven process. On the other hand, studying of binding processes at various pH indicates that ANC has a more enthalpic stability and probably has a more packed structure at pH=5 than that at pH=7.5 and 10.

Data analysis based on the Scatchard equation at various pH and temperatures shows a linear behavior with positive slope, indicating one set of binding sites.

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1. Introduction

Enzymes are highly selective catalysts, which operate under mild reaction conditions. Such properties make enzymes potentially attractive in synthetic chemistry, especially in the synthesis of natural products, pharmaceuticals, fine chemicals and food ingredients. Cellulases are enzymes that catalyze the hydrolysis of the β-1,4-glycosidic linkages in cellulose, the most abundant biopolymer. The term “cellulase” includes enzymes that have been classed into 13 structural families, as defined on the basis of protein sequence comparisons [1]. These enzymes can have widely different activities on cellulose substrates. Varieties of microorganisms secrete these enzymes either individually or associated in a macromolecular complex referred to as the cellulosome. These different cellulases, acting synergistically, catalyze the complete hydrolysis of cellulose to glucose, which, under anaerobic conditions, is a highly fermentable fuel product. The ecological and economic advantages of plant biomass conversion have spawned considerable interest in the study and eventual use of recombinant cellulolytic complexes.

Much is known about the catalytic mechanisms of cellulases (and glycoside hydrolases in general), and these enzymes have been extensively studied by steady-state kinetics and mutagenesis [2,3]. However, there are few papers in the literature describing thermodynamic aspects of cellulase, especially on the interaction between cellulase and small molecules such as surfactants.

The aim of this paper is to describe the development of a method for the study of interaction between a cationic surfactant and a cellulase to understand the relationships between structure and functionality of enzymes.

Interactions between surfactants and proteins have been extensively studied [4–13], since many industrial, biological, pharmaceutical and cosmetic products contain both proteins and...
surfactants. It is generally accepted that binding of ionic surfactant molecules to proteins disrupts the native structure of most globular proteins [4,14]. For general aspects of the interactions between ionic surfactants and water soluble-proteins, sodium dodecyl sulfate (SDS) and bovine serum albumin (BSA) are often used as an archetype type [15–19]. Another protein which also has been frequently studied is lysozyme. In an early case, apart from SDS interaction [19–21], other surfactants such as n-alkylsulfates [22] and n-alkyltrimethylammonium salts [23,24] are also studied. The binding of surfactant to the ionic sites has been observed. Further binding occurs by hydrophobic cooperative interactions. Hence, ionic surfactants interact with proteins through a combination of electrostatic and hydrophobic forces.

Enzymatic and chemical properties of Aspergillus niger cellulase (ANC) have been studied extensively [25–29], but its physical and chemical denaturation from the thermodynamic point of view are still largely undetermined. In the present work, we report a study of the aqueous solution behavior of the surfactant. The CTAB used in the present work was originally constructed to investigate the aqueous solution behavior of the surfactant. The membrane ion selective electrode (MISE) selective to cellulase (ANC), obtained from Fluka, was used as received. Calculations were made assuming a molecular weight of 35,000 D for ANC. CTAB, 98% purity provided by Aldrich Chemical Co., was purified by repeated crystallization from an acetone/water mixture. All solutions were prepared with double distilled water. All measurements were carried out in 50 mM sodium phosphate buffer solution.

2. Experimental

2.1. Materials

Aspergillus niger cellulase (ANC), obtained from Fluka, was used as received. Calculations were made assuming a molecular weight of 35,000 D for ANC. CTAB, 98% purity provided by Aldrich Chemical Co., was purified by repeated crystallization from an acetone/water mixture. All solutions were prepared with double distilled water. All measurements were carried out in 50 mM sodium phosphate buffer solution.

2.2. Method

The membrane ion selective electrode (MISE) selective to CTAB used in the present work was originally constructed to investigate the aqueous solution behavior of the surfactant. The procedures used to construct these MISE are now well documented [32–36].

The ion selective electrode sensitive to surfactant ion was used for the measurement of the free concentration of surfactant ions, [S]f, in equilibrium with surfactant–enzyme complexes at different conditions. The emf measurements of the surfactant selective electrode were measured relative to a commercial sodium ion electrode (Methrohm 60501–100). In all experiments, the temperature was controlled to within ±0.1 °C by circulating thermostated water (Optima 730) through the jacketed glass cell. The sample solution was continuously stirred using a magnetic stirrer.

At surfactant concentrations below the critical micelle concentration (cmc), the surfactant was dissociated completely. Therefore, the logarithm of the concentration of surfactant against the emf gives a Nernstian slope. Obviously, the potential of the electrode should be measured relative to the reference electrode. The cell configuration used was

Ag|AgBr|Internal solution|PVC membrane|Test solution |Reference electrode

The following equations can be written for different electrode potentials, according to the Nernst equation

\[ E_{S^+} = E_S^\circ + \frac{RT}{F} \ln a_{S^+} \]  
\[ E_{Na^+} = E_{Na^+}^\circ + \frac{RT}{F} \ln a_{Na^+} \]

where \( T, R, F, a_{S^+}, \) and \( a_{Na^+} \) are absolute temperature, gas constant, Faraday’s constant, activity of surfactant ion, and activity of sodium ion, respectively. \( E_{Na^+}, E_{S^+}, E_{Na^+}^\circ \), and \( E_{S^+}^\circ \) indicate the sodium, surfactant and corresponding standard electrode potentials, respectively.

The potential of each electrode depends upon the logarithm of the activity of the surfactant and sodium ions. In this way, cells without a liquid junction were constructed which respond to two ionic species concentrations, namely the surfactant monomer ion, \([S]_f\), and the co-ion (where \( Na^+ \)), \([C]_s\), comes from the backing electrolyte.

The electrochemical cell can be considered to be between the surfactant electrode and the sodium electrode as a reference electrode

\[ E_{cell} = E_{S^+} - E_{Na^+} \]

where \( E_{cell} \) is the potential of the electrochemical cell between surfactant electrode and sodium electrode.

The monomer concentration of the surfactant ion can be determined below and above the cmc using the following equations

\[ E_{cell} = E_{S^+}/Na^+ + 2.303 \frac{RT}{F} \log \left( \frac{[S]_f \gamma_{S^+}}{[C]_s \gamma_{Na^+}} \right) \]

at constant sodium ion concentration, and with the assumption that \( \gamma_{Na^+} \approx \gamma_{S^+} \) or \( \gamma_{Na^+} \approx 1 \) at low concentration of surfactant which applies to this experiment, leads immediately to

\[ E_{cell} = E_{S^+}/Na^+ + 2.303 \frac{RT}{F} \log [S]_f \]

where

\[ E_{S^+}/Na^+ = E_{S^+}/Na^+ - 2.303 \frac{RT}{F} \log [C]_s \]

Since the surfactant is dissociated completely into ions below the cmc, the plot of \( E_{cell} \) against \( \log [S]_f \) obeys Nernstian behavior.
Hence, the slope of the line is referred to \((\pm 2.303 \text{ RT}/F)\) and the intercept, \(E_{\text{S+/Na+}}\). This calibration line can be used for the determination of monomer surfactant ion concentration above the cmc by adjusting data on the calibration line. A least mean squares method was used for determination of the slope, \(2.303 \text{ RT}/F\), and intercept, \(E_{\text{S+/Na+}}\), for each set of data.

3. Results and discussion

Fig. 1 shows the plot of emf versus the logarithm of total surfactant concentration, \(\log[S]_t\), in the absence of ANC at specified experimental conditions. It is obvious that at low concentrations of surfactant (below the cmc), the emf is directly proportional to \(\log[S]_t\) with a Nernstian slope (57–60 mV). However, at higher concentrations, the resulting plots show a distinct break at the cmc. An increase in surfactant concentration causes a decrease in \([S]_t\) in the concentration range above the cmc. The decrease in monomer surfactant concentration above the cmc was confirmed by Hall’s theory in 1981 [37]. However, many workers have assumed that the monomer concentration is constant above the cmc [38,39]. The cmc value obtained for CTAB is \(9.2 \times 10^{-4}\), which is in good agreement with the literature value [40].

The changes in the emf of the buffer solutions in the presence of ANC on the addition of CTAB solution, at various temperatures have been shown in Figs. 2 and 3. The calibration curve clearly shows the excellent performance of the CTAB-specific membrane electrode. The deviation from the calibration curve in the presence of ANC allows us to calculate the amount of surfactant bound to ANC.

From the calculation of the concentration of free CTAB, \([S]_f\), by Eq. (5) and subtracting from the total concentration of CTAB, \([S]_t\), the concentration of bound CTAB, \([S]_b\) was
calculated. By considering the fact that the emf is reduced in the presence of ANC, the amount of surfactant bound to ANC can be calculated.

The average number of surfactant molecules bound per ANC molecule has been calculated as

$$m = \frac{S_i}{C_p t} - \frac{S_i}{C_{138} f} C_p \quad (7)$$

where $C_p$ is the total concentration of ANC.

Figs. 4 and 5 are the binding isotherms of ANC–CTAB interaction which shows the number of CTAB ions bound per molecule of ANC ($\nu$) as a function of $\log [S_i]$, at the specified conditions. As has been shown, affinity of ANC for binding was increased with increasing of temperature, a result of unfolding of enzyme. At higher temperatures, proteins undergo an unfolding process and the binding sites are more available for binding, so the affinity for binding was increased. On the other hand, the binding isotherms show that the ANC is more stable at pH=5, so at this pH the interaction occurred at higher concentration of CTAB. By the way, ANC is more unstable at pH=7.5 and interaction started at lower concentration of surfactant.

Calculation of the apparent binding constant, $K_{app}$, can be applied to the entire binding isotherm. This is based on the Wyman binding potential energy [41]. The Wyman binding potential, $\Pi_{\nu}$, has the property that

$$v_i = m \frac{\partial \Pi_{\nu}}{\partial \mu_i} T , P, \mu_{i=j} \quad (8)$$

where $v_i$ and $\mu_i$ are the number of average bound ligands per macromolecule and chemical potential of component $i$. 

Fig. 5. Binding isotherms for interaction between ANC and CTAB at $t=30$ °C and various pH: (●) 5, (■) 7.5, (▲) 10.

Fig. 6. Variation of Gibbs free energy of binding per mole of CTAB on interaction with ANC as a function of $\nu$: (■) 30 °C, (●) 35 °C, (▲) 40 °C; at pH=7.5.

Fig. 7. Plot of $\Delta H_{\nu}$ versus $\nu$ for interaction between CTAB and ANC at pH=7.5.

Fig. 8. Scatchard plot for binding of CTAB to ANC at pH=7.5 and various temperatures: (■) 30 °C, (●) 35 °C, (▲) 40 °C.
respective. On the basis of Eq. (8), at constant pressure, temperature and activities of all other components except ligand, we can write

$$\Pi_i = RT \int_0^{\ln[S]_f} v \, d \ln[S]_f$$

(9)

The binding potential is calculated from the area under the binding isotherm according to Eq. (9). This is related to an apparent overall macroscopic binding constant, $K_{app,v}$, as follows [42]

$$\Pi_i = RT \ln \left( 1 + K_{app,v} [S]_f^v \right)$$

(10)

where $K_{app,v}$ is the apparent macroscopic binding constant for the $v$th association reaction. Values of $K_{app,v}$ were determined by application of Eqs. (9) and (10), and used to determine the values of the Gibbs free energy of binding per mole of surfactant, $\Delta G_v$,

$$\Delta G_v = -\frac{RT}{v} \ln K_{app,v}$$

(11)

Fig. 6 shows variation of $\Delta G_v$ versus $v$ for the binding of CTAB to ANC at various temperatures. It is obvious that the affinity of binding reaches a minimum and maintains constant after a specific value of $v$. As has been shown in Fig. 6, affinity of binding was increased with increasing $v$. The limiting value of $\Delta G_v$ (approximately $-9.5 \text{ kJ mol}^{-1}$) can be predominantly related to hydrophobic binding. However, the variation of $\Delta G_v$ with respect to $v$ does not show the trends expected from the cooperativity coefficients.

The values of $\Delta G_v$ are more negative at higher temperatures, which represents the endothermic and essentially entropy driven process. With respect to the entropic nature of hydrophobic and enthalpic nature of electrostatic interactions, this observation represents the more predominant role of hydrophobic interactions in the overall process [43].

Fig. 9. Scatchard plot for binding of CTAB to ANC at 30 °C and various pH: (●) 5, (■) 7.5, (▲) 10.

The enthalpy of binding, $\Delta H_v$, of CTAB to ANC was obtained from the temperature dependency of the binding constant ($K_{app}$) using the van’t Hoff relation [44]

$$\Delta H_v = -R \frac{d(\ln K_{app})}{d(1/T)}$$

(12)

where $\Delta H_v$ is an approximate value of $\Delta H$ per mole of CTAB; $\Delta H_v = \Delta H/v$. The plot of $\Delta H_v$ as a function of $v$ is shown in Fig. 7. Fig. 7 shows an endothermic tendency for the interaction process between CTAB and ANC. The $\Delta H_v$ value increased with increasing $v$ indicating a shift of interaction to more hydrophobic interaction. As unfolding region extents, the unfolding enthalpy increases, i.e. heat content of the three-dimensional structure of ANC after binding of the surfactant is higher than that before binding.

Comparison between binding isotherms at different pH (see Fig. 5) show that ANC has a more enthalpic stability and probably has a more packed structure at pH=5 than that at pH=7.5 and 10.

The Scatchard plot, i.e. $v/[S]_f$ versus $v$, was obtained from Scatchard equation, which can be used for analyzing the systems with one binding set [45,46]. The linear Scatchard plot indicates the identical and independent set of sites, whereas the non-linear curves (upward or downward concave) indicate the non-identical and dependent set of binding sites [47]. The Scatchard plots for binding of CTAB to ANC at different pH and temperatures are shown in Figs. 8 and 9. All Scatchard plots show a linear behavior with positive slope, indicating non-identical binding sites.

4. Conclusions

Analyses of binding data for the interaction of CTAB with ANC suggest a system with one set of binding sites. The limiting values of $\Delta G_v$ at high concentrations of surfactants are approximately equal to $\Delta G_{micelle}$, which indicates that the structure of the ANC-surfactant complex and the related micelle are similar.

The endothermicity of the process also indicates that the hydrophobic interactions play an important role in the ANC-surfactant binding, whereas electrostatic interactions play only a minor one.

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