Journal of Physical and Theoretical Chemistry
of Islamic Azad University of Iran. 6 (4) 231-236: Winter 2010
(J.Phys.Theor.Chem. IAU Iran)
ISSN: 1735-2126

Potentiometric Study on the Interaction of Hexadecyl Trimethyl Ammonium Bromide (HTAB) with Urease Enzyme

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ABSTRACT

In this research, the interaction of hexadecyl trimethyl ammonium bromide (HTAB) with enzyme urease has been investigated comprehensively at different experimental conditions such as ionic strength, protein concentration using inn selective membrane electrode of surfactants. The obtained binding isotherms from potentiometric studies have been analyzed by different theories such as Wyman binding potential, Scatchard diagram, binding capacity concept and Hill equation. The results indicate the aggregation of urease at concentrations more than 1 mg/ml of protein. Increasing the ionic strength to 1 mM, causes to decrease the interaction with urease but increasing the ionic strength to more than 1 mM again causes to increase the interaction. This issue can be due to stability of urease at ionic streogth of 1 mM. Increasing the concentration of urease to 3 mM causes to gradual and regular decreasing of interaction and at higher concentrations, the intense increase in interaction is resulted. Increasing pH from 6.5 to 9.7 does not create grent changes at binding isotherms that is due to slight change of dissociation degree of acidic and basic groups and third structure of urease at this limit. In all studied cases in comparison with similar case, it shows stronger interaction with urease. This issue is justifiable according to longer hydrocarboa tail that increases its hydrophobic property that indientes the special role of hydrophobic interactions in interactions proecss of ionic surfactants with proteins.

Keywords: Urease; Hexsdecyl trimethyl ammonium bromide (IITAB); Ion selective electrode; Bindiag isotherm

INTRODUCTION

Using the method of coastructing the ion selective electrode which is sensitive to surfactant, the concentratiun of surfactant in iateraction with urease enzyme can he determined. This method helps us to obtain acceptable results in order to compute and analyzing the thermodynamic data. In this research, stability and thermodynamic properties of urease enzyme has been investigated. In this order at first we prepared the membrane ion selective electrode of surfactant, so an electrochemical cell was designed for attaining the potentiometric data of surfactant binding to urease. Potentiometry reply is used to attain the bindiag isotherms for binding of surfactant to urease

Using calculated amounts nf Gibh's free energy change of binding (ΔG_r), we will be ahle to discuss about thermodynamic of binding.

Investigation the effect of environmental conditions such as pH, ionic strength, enzyme concentration and presence of urea as a chemical denaturant on hinding pmcess are important purposes of this research. Finally usiog hinding data and calculating the Wyman binding potential (π), hinding capacity (θ) and the shape of Scatchard plots, we analyze the urease structure in order to determine the number of hinding site sets, affinity of each site and the

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number of bound places in each binding set at any specified experimental conditions. The effect of HTAB on the urease enzyme is investigated using potentiometry technique and the results were analyzed on basis of binding mechanism and Scatchard viewpoints of urease.

EXPERIMENTAL

Materials

Urease enzyme from Jack beam with EC code of (EC, 3. 5. 1, 5), triphosphate, carboxylate polyvinyl chloride (PVC) with high molecular mass, hexadecyl trimethyl ammonium bromide (HTAB), THF, acetone, nitric acid, hydrochloridric acid, sodium bromide, pentaoxide dephosphorus, ethanol, sodium hydroxide and urea were obtained from Merck. Dcoetyle phetalate (DDP) was obtained from Aldrich. Silver wire and reference electrode of sodium was obtained from Metrohm Company.

Equipments

All potentiometry and pH-metry determinations were carried out on ΩMetrohm-744 pH-meter and potentiometer. Because of electrode sensitivity to temperature, all experimeots were done under the temperature controlling of apparatus. The HT-202 Heater-stirrer was used to homogenize the solutions.

METHODS

Preparing the membrane and ion selective electrode of surfactant

In order to obtain a suitable membrane for making selective electrodes that act reversible for cationic surfactant ions of HTAB, we used carboxylate PVC with high molecular mass which would be activated by surfactant cations. PVC (0.5g) was dissolved in THF (20mL). This solution was added dropwise to the 50mL of surfactant solution (3 mM) and was stirred calmly to attain a fibrous precipitate that was filtered and washed by double distilled water, then was put on a watch glass and transferred into a desiccator containing P_2D_5, to he desiccated completely (complete desiccation took 24 hours). In order to prepare plasticizer salutian, 0.18 g (DOP) was dissolved in 3-4 mL THF solvent. 0.12 g of desiceated membrane was added to DOP solution. It took 4-6 hours to obtain a limpid and homogenized gel in effect of vaporizing the THF.

In next stage, glass tubes should he prepared, so we used glass tubes with diameter of 5 mm and length of 10 cm. We used emery in order to obtain a complete smoothness on the surface of glass tubes, and theo they were washed and dried for binding the membrane to them. For preventing the air current interference and smoothing the basic layer thickness of membrane, we closed the tube mouth by forefinger, and then put it into the membrane gel. After emitting. It was put vertically to expose to the air for at least 12 hours.

Coating the surface of silver wire

The surface of silver wire should be coated by precipitate of silver bromide. We used a saturated solution of sodium bromide and a dilute solution of nitric acid. At first stage, the surface of silver wire was cleaned by emery and was washed with water and ethanol, and then 3-4 cm of wire was entered into the nitric acid solution. Surface of silver wire was oxidized in a short time less than 1 minute, so a thin layer of Ag^- ions were formed on the wire surface that composed with bromide ions after transferring to the saturated solution of sodium bromide and precipitated again on the surface of the silver wire.

Conditinning solution

This solution is 1mM related to the surfactant and 0.1mM related to the NaBr. The prepared glass electrode in previous stage was put in solution from both inner and outer part. It took 24 hours to prepare the membrane surface of electrode. After these stages, with entering a coated silver wire into the standard solution inside the tube we can use the surfactant electrode for basic determinations.

Determination method

All potentiometric experiments were carried out using a 10 mL beaker as determination cell. Initial tests were done on electrode. A 5 mL buffer solution of NaBr (10^{-4}M) was placed in the cell and ion selective electrode of surfactant was put on the solution next to a refereoce

electrode of sodium. The connective wires of electrode were connected to the potentiometer. Using micropipette, equal volumes of 10 ∝L of surfactant were added to test cells and emf was recorded. Finally, the amounts of obtained emf were plotted versus log $[s]_f$. Linearity of curves with Nernst slope indicates the correctness of the electrode reply. After confiding in correct reply, we did the experiment in presence of a given concentration of urease. The method of experiment is similar to the previous stage but the experiment was carried out in presence of NaBr (10^{-5}M) and also other conditions such as ionic strength or pH were different from previous stage.

Investigating the effect of urease concentration on the surfactant binding

We chose concentrations of 0.5, 1.1, 3.2 and 4.1 mg/mL of urease, pH = 6.5, NaBr (0.1 mM).

Investigating the effect of pH on the interaction of surfactant with urease

From previous section, we concluded that concentration of 1mg/mL is the best concentration for quantitative experiments. In this section, experiments were carried out at pH=6.5 and 9.5. In order to adjust the pH, we used concentrated solution of NaOH and HCl with concentration of (0.5M).

Investigating the effect of ionic strength on the binding of surfactant to urease

In this section, the solutions with constant concentrations of urease enzyme and at different ionic strength were prepared. In this regard the concentrations of $10^{-7}$and 10^{-2} M of NaBr was chosed. All experiments were carried out at pH = 6.5.

Investigating the effect of chemical denaturant

Urea is one of the important denaturants of proteins. Urea and hydrochloride guanidine cause to unfolding the protein through hydrogen bond, which is stronger than water-protein binding. In other hand urea solution is not stable so decomposes to ammonium and cyanate ions. Urea solution should be freshly prepared and used due to interaction between cyanate ions and urease enzyme.

RESULTS AND DISCUSSION

Designed electrochemical cell for determining the surfactant concentration, contains a reference sodium electrode and an ion selective electrode sensitive to surfactant. A special volume of buffer solution consists of NaBr (10^{-4}M) and protein (1mg/mL) is used. After turning the potentiometer on, absolute volumes of surfactant were added gradually and potential difference was recorded. The obtained information will be investigated using Excel software. The plot of emf versus logarithm of surfactant concentration shows that in starting point, that binding process has not been occurred, potentiometer reply is nearly independent of protein presence. Relation of potential to surfactant concentration is expressed by equation stated as below:

$$Emf = E^\circ + m \log[s]_f \qquad (1)$$

where Emf is, obtained potential from potentiometer, E° is intercept of plot in initial part and m is slope, which is attained between 57 to 61mv. Concentration of free surfactant is calculated using equation mentioned above. We can determine the number of bound surfactant moles to enzyme from difference of total and free surfactant concentration. Then we can attain the proportion of average bound surfactant moles to total existent enzyme moles (υ), and calculate the binding potential, appearance binding constant and molar Gibb's free energy change from binding isotherms plot.

Calibration plot of potentiometer reply

The plot of emf variation versus log $[s]_f$ at various pH shows three distinct regions that are shown in Fig.1. Initial part of plot is a straight line with Nernstian slope, corresponds to very low concentration range of surfactant that the binding has not been started. This part is used as standard reply and obtained equation will be basic reply of electrode for next parts. The middle part is the start point of binding process and forming the surfactant – protein complex. The end part is the sign of approaching to the critical micelle concentration (CMC) region, so with increasing the monomer concentration in solution and aggregation incidence, reduction of concentration in solution or reduction of potential difference will be observed actually.

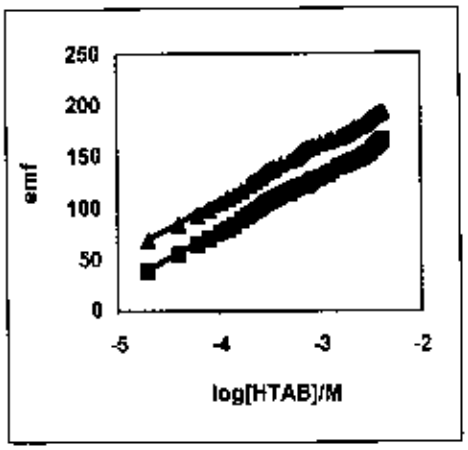

Fig. 1. Variations of emf versus log [HTAB] at pH=6.5 (■) and 9.5 (▲), [urease] =1mg/ml, t=25^{0}C, [NaBr] = 0.1 mM.

Analysis and Interpretation of binding isotherms

Fig. 2 shows the binding isotherms fnr interaction of HTAB with urease enzyme at different concentrations of protein. It seems that these curves in the limit of measurement uncertainty conform on each other io concentrations of 1 and 2 mg/mL. and at higher concentration, curves show relatively high difference and in a special concentration of HTAB, u tends to fewer amounts This manner is duc to aggregation phenomenon at higher concentration. In fact enzyme aggregation increases upon increasing the concentration.

Plot shnws that with increasing of urease enzyme aggregation, binding of surfactant to urease (υ_{app}) decreases, so we can claim that at higher concentration of urease. resistance of urease to HTAB increases duc to urease aggregation. Based on these results, concentration of 1mg/mL of urease is the most suitable concentration; because it is the highest caocentration that aggregation phenomenon has not been occurred and has the most precision.

Fig. 3 shows binding isotherms for interaction of HTAB with urease at different pH. Negative charge density on urease enzyme increases upon increasing the pH, so interaction of the cationic surfactant with urease increases. Shifting of binding isotherms to fewer concentrations at higher pH indicates that electrostatic effects increase upon increasing the pH. These results confirm the ohtained results from previous investigations about urease structure.

Fig. 4 shows the effect of ionic strength on interaction of HTAB and urease. At first with increasing the ionic strength from 10^{-4} to 10^{-3}M, the binding isotherm plots shift to higher concentration of surfactant. It means that with increasing the innic strength, the role of electrnstatic forces decrease so interactinn decreased and with increasing the innic strength from 10^{-2} tn 10^{-1} M, the role nf hydrophobic forces overcnme to electrnstatic fnrces and interaction increased.

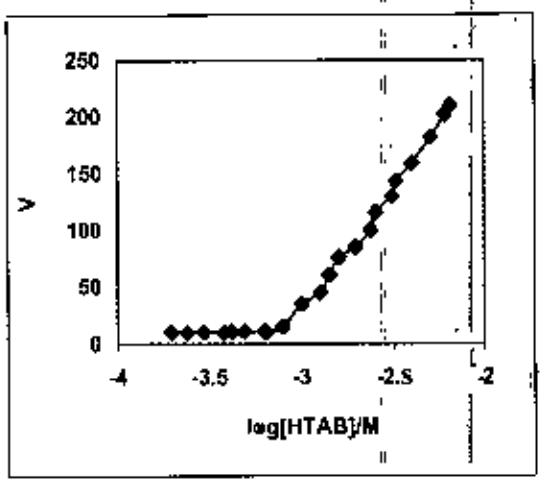

Fig. 2. The binding isotherm for interaction of HTAB with urease at pH=6.5. t=25^{0}C, [NaBr] =0.1mM.

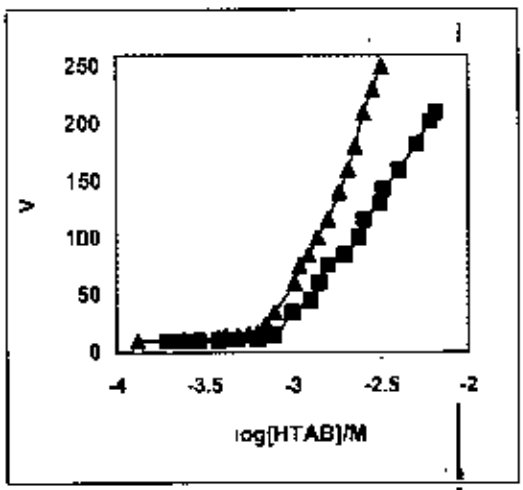

Fig. 3. The binding isotherms for interaction of HTAB with urease at pH = 6.5(■) and 9.5(▲), t=25^{0}C and [NaBr] = 0.1 mM.

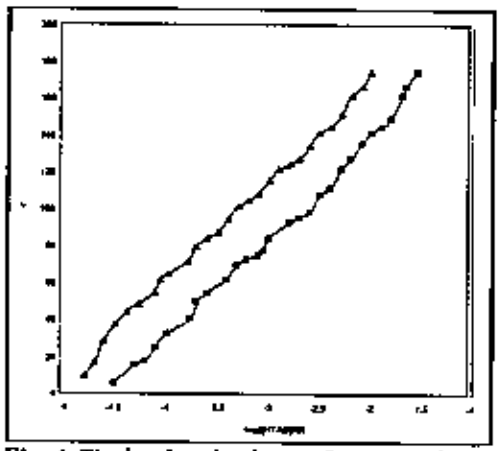

Fig. 4. The binding isotherms for interaction of HTAB with urease at various concentration of NaBr. 0.1 mM(■), 0.01 mM(▲), pH=6 5 and t=25°C.

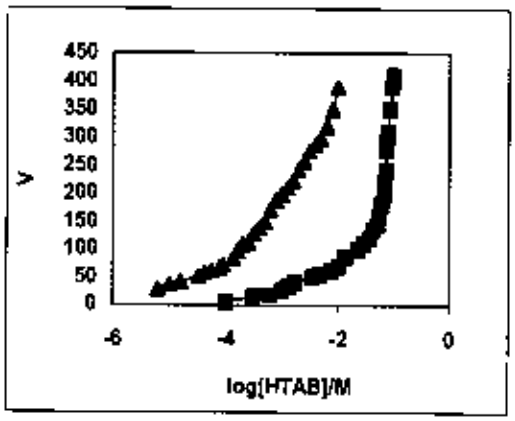

Fig.5. The binding isotherms for interaction of HTAB with urease at various concentration of urease, 1M (▲), 3M (■), pH=6.5, t=25°C and [NaBr]=0.1mM.

Fig.5 shows that binding isotherms are placed in higher states in absence of urea compare to binding isotherms with urea concentration of 3M. In these states, concentration of urea is not enough for denaturing of urease, so cause to decrease hydrophobic interactions and decreases the binding of HTAB to urease. Slight difference and shifting are due to the hydrophobic tails difference of HTAB, so interactions of HTAB are more predominant because of longer hydrophobic tail of HTAB. So curves appear at fewer concentrations.

Overlapping of binding isotherms at concentrations higher than 5M indicates the denaturation of urease enzyme in this range of urea cancentration. Binding isotherms have been shifted towards the fewer concentrations because of unfolding of urease enzyme and destruction of its compact structure and increasing the connection surface and probability of connecting of surfactant to binding sites of urease enzyme.

The variations of Gibbs free energy

ΔG_v variations about HTAB at pH=6.5 and 9.5 in the beginning of binding is more and decreases gradually. This issue can be due to the predominant role of electrostatic interactions at the beginning and hydrophobic interactions at the end of the binding process of HTAB. On the other hand, decreasing of ΔG_v at pH=9.5 with respect to pH=6.5 can be due to the more effectiveness of statistical effects role at ΔG_v values that is a macroscopic quantity. Investigating the Fig. 6 indicates that ionization difference in ionized acidic and basic groups in urease enzyme in these two pH is low because the binding amount in two cases are similar. Fig 6 shows ΔG_v variations versus log [HTAB] at various ionic strengths. At first with increasing the ionic strength from 10^{-4} to 10^{-3} M, interaction is decreased but with more increasing of ionic strength, the hydrophobic forces show more predominant role in interaction. According to related binding isotherms, at first with increasing the ionic strength from 10^{-5} to 10^{-4}M, the curve shift to right hand that indicates the decrease of interaction and then with increasing the ionic strength to 10^{-3} and 10^{-2} M, the curve shift to left hand that indicates increasing of binding affinity. At the end we can claim that urease has the most stability at ionic strength of 10^{-4} M.

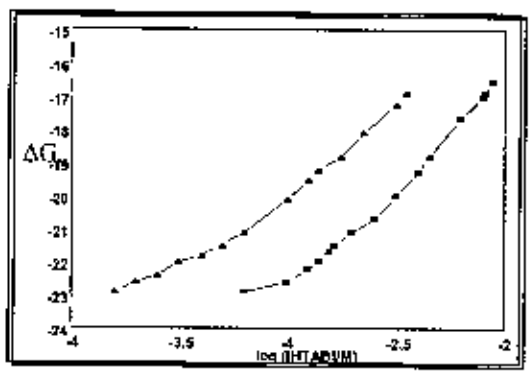

Fig. 6. The variation of ΔG_v versus log ([HTAB]/M) at various ionic strengths, 0.1 mM (▲), 0.01 mM (■) at pH=6.5 and t=25°C.

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