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ORIGINAL ARTICLE **OPEN ACCESS**

Binding Mechanism of Dodecyl Trimethylammonium Bromide (DTAB) with Lysozyme Hydrolytic Enzyme at Various Surfactant Concentrations

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ABSTRACT

Lysozyme is a hydrolytic enzyme which has been purified from cells, secretions and tissues of virtually all living organisms and viruses. Lysozyme is a lytic enzyme found in many natural systems. It is a small and stable enzyme whose were completely analyzed dimensional structure and sequence. Binding of dodecyl trimethyl ammonium bromide (DTAB) with Lysozyme at various surfactant concentrations and at various pH, is studied at 37℃ by equilibrium dialysis technique. The binding isotherms at pH values of 3.2, 7.2 and 10 show cooperative binding at all surfactant concentrations and the binding ratios increase with increasing of pH. However; the increasing of binding ratio from pH=3.2 to 7.2 is more than pH= 7.2 to 10, which may be due to the little change in the ionization state of titration groups of Lysozyme at this pH range. The Gibb's free energy change ΔG_v calculated on the basis of Wyman binding potential concept and intrinsic binding free energy, ΔG_v calculated on the basis of Hill, Tanford and Scatchard equations. The best fitting of binding data at all of the studied pH have been obtained in the Hill equation with two sets of binding sites. The existence of a linear relation between both kind of Gibb's free energy and pH at the same free concentration of DTAB and at the same binding ratio have been examined. The best linear relations have been obtained for ΔG_v of the first binding set and ΔG_v for the second binding set, the other parameters have not shown a specified linear relation. On the basis of these linear relations, the contribution of electrostatic and hydrophobic forces in Gibb's free energy has been separated. The results represented the more contribution of hydrophobic interaction in the Gibb's free energy in the first binding set. The results of this analysis represented the contribution of electrostatic and hydrophobic forces in free energy of interaction and the amount of cooperatives in the process of binding.
Keywords: Lysozyme Protein, Surfactant, Equilibrium, Dialysis, Surfactant

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INTRODUCTION

Surfactants are groups of materials that contain a polar and hydrophilic part and a nonpolar and hydrophobic part. Because of this characteristic, surfactants show properties like decreasing the Surface tension; forming the micelle and dissolving of nonpolar compounds. Changing the environmental conditions such as temperature, ionic strength, pH and etc, change the surface activity of surfactants due to changing the hydrophobic amount.

Interaction between surfactants and globular proteins has been investigated through physical methods vastly [1].High consumption of detergents that surfactants are major part of them causes to entering them to environment and polluting the vital systems, so study of the interaction between surfactants and proteins is important for understanding the mechanism of pollution. In addition understanding the structure of complex of protein with surfactant can represents useful information about protein-lipid, which is an unknown phenomenon. The denaturizing of the protein is a key study for obtaining structural information. Most of the protein denaturation studies by surfactants have been done using anionic surfactants like sodium n-dodecyl sulfate (SDS) [2].

There are also some studies using cationic surfactants. The study of the nature of the interaction between protein and surfactant provides insight into action of surfactant as denaturant. Proteins and surfactants both contain a balanced proportion of hydrophilic and hydrophobic groups. It has been suggested that the

interaction between ionic surfactants and proteins involve initial binding of the surfactant molecules to charged groups of opposite sign on the surface of the protein followed by more extensive hydrophobic interactions until the critical micelle concentration (CMC) of surfactant is reached [3]. Binding of some n-alkyl sulfates to Lysozyme have been investigated using equilibrium dialysis at 25°C by Jones. Also binding of cetyl trimethyl ammonium bromide (CTAB) and dodecyl trimethyl ammonium bromide (DTAB) to Lysozyme at various pH have been studied by Venkatappa [4].
He indicated that Gibb's free energy (ΔG) increases when pH and hydrocarbon chain length of surfactant increase. Our interest in this report is investigation of the interaction between dodecyl trimethyl ammonium bromide (DTAB) and Lysozyme, by equilibrium dialysis technique that can give us information about mechanism of interaction and role of different parameters and also relation of these parameters to hydrocarbon chain length [5].

EXPERIMENTAL

Materials and conditions

Lysozyme and other materials were obtained from Merck. Orange (II) dye, DTAB and Dialysis bag were obtained from Sigma. For preparing all solutions we used double distilled water.
UV-Vis Spectroscopy studies have been done at 37°C and equilibrium dialysis studies have been done at 37°C. We used phosphate buffer (5mM, pH=7.2) and glycine buffers (pH=3.2, pH=10 and I=0.125). In all calculations we considered Lysozyme with molecular weight of 14320 Dalton.

Preparing the dialysis bag

Dialysis bags were boiled in carbonate sodium (5% w/w) for 15 minutes, then were washed with distilled water for three times. Then put them in a solution of EDTA (0.5 Molar) for emitting the metallic ions.

Preparing the orange (II) dye

A 200 ml flask was charged with orange (II) dye (0.2943gr) and NaCl (2.6250gr), so we got a solution of $1.68*10^{-4}$ of dye in NaCl (0.1M)

Methods

Absorption titration of Lysozyme

Lysozyme has an absorption maximum wavelength at 281nm. A buffer of phosphate (2ml) was added to both quartz cells of UV spectrometer, and then we replaced solution of sample cell with 2ml solution of protein (0.3mg/ml) in same buffer. Absorption of solution was recorded at maximum wavelength. This method has been done according to the literature procedures.

Equilibrium dialysis

We took sixteen test tubes (1*7cm) and surfactant solutions (2ml) with different concentrations were placed in each test tube. Then a dialysis bag containing 2ml of protein with concentration of (1mg/ml) was put in each tube and their lids were closed firmly and equilibrated for over 96h in a water bath with a specified temperature. Then concentrations of surfactants in solutions were determined using calibration curve.

Measurement of (DTAB) by colorimetric method

We took some test tubes (15*1cm), and surfactant solutions (4ml) were placed in them. Then we added orange (II) dye (1ml) and chloroform (5ml) to each tube respectively, then transferred them to centrifuge tubes, and centrifuge them for 5 minutes, then separated water phase and determined the organic phase at 485nm. Constructing the variations of absorption versus surfactant moles in a sample solution give us calibrations curve. We have done this method according to the literature procedures [6].

Conductometry method

Using calibrated conductometer we can determine critical micelle concentration (CMC) of surfactant at 30°C. This amount of CMC for (DTAB) is about 12.

RESULTS AND DISCUSSION

We can obtain the average free energy change for binding of one mole of surfactant to protein from equation:

$$\Delta G_{Vi} = \frac{RT}{\nu_i} Ln \quad K_{app} \,, \nu_i$$

Where $K_{app,vi}$ is total apparent binding constant for v_i association reaction and v_i is number of average bound ligand per protein. According to the nature of surfactants and proteins, we consider two kind of electrostatic and hydrophobic forces [7].
Scatchard figures can be very inaudible and misleading for interpreting these systems, so they represented a method for estimating the number of sites and their properties and existence of two sets of site at the process of binding of surfactants to globular proteins. One of the common ways for analyzing

the binding data is fitting them to the Hill equation. We can write Hill equation for analyzing a system with two binding sets as equation stated below:

$$\nu = \frac{g_1 (K_{H1}[s])^{n_{H1}}}{1 + (K_{H1}[s])^{n_{H1}}} + \frac{g_2 (K_{H2}[s])^{n_{H2}}}{1 + (K_{H2}[s])^{n_{H2}}}$$

Where g_1, K_1, n_{H1} are the number of sites, Hill binding constant and Hill coefficient for the first binding set respectively, and g_2, K_2, n_{H2} are related parameters for second binding set. [s] is concentration of surfactant. ΔG_v is calculated based on Wyman binding potential concept [8].

Fitting binding data to the Hill equation shows that, at all pH, the best fitting have been obtained in the Hill equation with two sets of binding site see Table 1. Amount of parameters derived from linear fitting of ΔG_v of first binding set in a specified log[s] versus pH are tabulated in Table 2, and amount of parameters derived from linear fitting of ΔG_v from second binding set in a specified log[s] versus pH tabulated in Table3.

Table1. Parameters derived from fitting the binding data to the Hill equation with two sets of site

pH	g_1	K_{H1}	n_{H1}	g_2	K_{H2}	n_{H2}
3.2	14	317.10	2.09	34	104.30	8.25
7.2	14	569.40	1.36	33	178.80	5.28
10	14	475.50	1.51	38	238.40	1.69

Table2. Values of parameters derived from linear fitting of ΔG_v values of first set of site versus pH in a specified log [s]

Log [s]	Slope	Intercept	Correlation coefficient
-2.75	-0.162	-16.36	-0.85
-2.68	-0.138	-16.69	-0.79
-2.55	-0.193	-15.92	-0.95
-2.47	-0.237	-15.31	-0.99

Table3. Values of parameters derived from linear fitting of ΔG_v values of second sets of site versus pH in a specified log [s]

Log [s]	Slope	Intercept	Correlation coefficient
-2.311	0.006	-15.73	0.98
-2.291	0.008	-15.72	0.99
-2.271	0.118	-15.71	0.98
-2.253	0.025	-15.69	0.99
-2.234	0.130	-15.68	0.97

Scatchard diagrams for binding of (DTAB) to Lysozyme at pH values of 3.2, 7.2 and 10. Scatchard diagrams indicate two sets of binding site. At pH values of 10 and 3.2, Scatchard diagrams have a maximum and then a concave state that is due to positive cooperatively at second set of binding site, and Scatchard diagram at pH=7.2 indicates a system with a more positive cooperative at second set of binding site.

According to the mentioned issues we can conclude that binding affinity increases with pH, which is due to the increasing of negative charge density on the Lysozyme surface. Also low difference of affinity at pH=7.2-10 indicates complete ionization of groups at pH= 7.2. This matter helps us to separate the contribution of electrostatic and hydrophobic forces. Amount of electrostatic forces in comparison with hydrophobic forces indicates more contribution of hydrophobic forces at the first binding set. Amounts of electrostatic forces increase with increasing the pH and negative charge density on the Lysozyme surface.

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