A Thermodynamic Study of the Binding of Human Serum Albumin onto *N,N′*-Diethylaminoethyl Dextran Microbeads

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ABSTRACT: Adsorption of proteins on solid surfaces is widely studied because of its importance in various biotechnological, medical, and technical applications, e.g., biosensors, cardiovascular implants, and chromatography. Adsorption thermodynamics has been studied on the microbeads of *N,N′*-diethylaminoethyl (DEAE) Dextran anion exchanger for the human serum albumin (HSA) at 25, 30, 35, 40, and 45°C. As a result, some thermodynamic parameters like Freundlich constants, thermodynamic equilibrium constant (K_D), standard free energy changes (ΔG_{assoc}), standard entropy changes (ΔS_{assoc}), and standard enthalpy change (ΔH_{assoc}) have been evaluated. Using the linear Van't Hoff plot, ΔH_{assoc} value of the system for the interaction of bovine serum albumin (BSA)-adsorbed crosslinked DEAE dextran microbeads was determined as 20.650 kJ/mol. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 3942–3947, 2006

Key words: UV–vis spectroscopy; polysaccharides; adsorption; human serum albumin (HSA); ion-exchangers; binding energy

INTRODUCTION

The chromatographic separation of proteins is important not only for analysis but also in such large-scale industries as the food and the drug industry. Bioaffinity chromatography is a highly specific separation technique used for the isolation and purification of biomolecules. However, some limitations such as biological instability, leakage desorption conditions, and cost confine its application. A number of ion exchangers for protein separation have been developed for use in analysis, and a large number of chromatograms have been presented to show that they are useful for protein separation. When these ion exchangers are applied to large-scale chromatographic separation, it is necessary to investigate the equilibrium, kinetics, and dynamics in detail. Dextran-based polyelectrolyte displacers were successfully employed for the displacement purification of proteins in ion-exchange displacement systems.[1] As these types of separations become more common and higher-quality purifications are demanded, a method to optimize the separations with the minimum amount of experimentation is needed. At present no such method of predicting protein separations exits.[2,3]

Yamamoto et al.[4] showed that the adsorption of bovine serum albumin (BSA) on crosslinked *N,N′*-diethylaminoethyl (DEAE) dextran (a weakly basic dextran-type ion exchanger) decreases with decreasing pH. Tsou and Graham[5] showed the effects of the concentration of NaCl (c_e) in the BSA solution on the isotherm for adsorption of BSA on DEAE at pH = 6.9.[6] They showed that the isotherm for $c_e = 0$ is much more favorable than that for $c_e = 1\%$. However, these reports are fragmentary and systematic experimental investigations for adsorption isotherms of protein have not been reported. Yoshida et al. [7] presented experimental equilibrium isotherms of adsorption of BSA on crosslinked DEAE dextran ion exchanger using Na^+ and Cl^- ions.

The thermodynamics and dynamics of interfacial layers have gained a large interest in interfacial research. An accurate description of the thermodynamics of adsorption layers at liquid interfaces is a vital prerequisite for a quantitative understanding of the equilibrium or any nonequilibrium process going on at the surface of liquids or at the interface between two liquids. Adsorption isotherm models can be derived for a surface layer model in which the molecules of the surfactant and the solvent from which the adsorption takes place obey two conditions: (i) they do not interact with each other; and (ii) they occupy equal areas. In a number of cases, deviations from Langmuir be-

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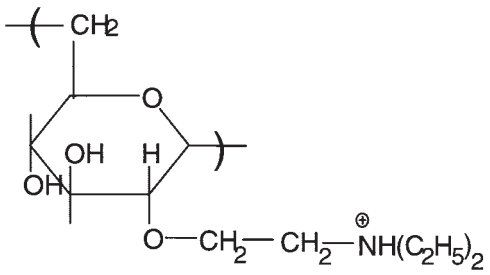
havior can be explained by relaxing the former condition, i.e., by accounting for interaction between the adsorbed molecules; in other cases, such deviations have been related to differences in molecular area.[8,9]

Human serum albumin (HSA) is the most abundant protein in blood plasma. They have many important physiological functions which contribute significantly to colloid osmotic pressure and aid in the transport, distribution and metabolism of many endogenous and exogenous substances including bile acids, bilirubin, long-chain fatty acids, aminoacids (notably tryptophan, thyroxine and cysteine) steroids (progesteron, testosterone, aldosterone, cortisol), metal ions such as copper, zinc, calcium, magnesium chloride and numerous pharmaceuticals. In recent years, microporous spheres were modified and various affinity materials were used as alternative supports for protein separation. Several studies on HSA binding of small molecules, particularly fatty acids and surfactants, based on different spectroscopic techniques have been reported aiming to clarify the nature of the interaction with the ligand as a function of ionic strength, temperature and viscosity, as well as the nature of fatty acids or anionic surfactants.

Adsorption of HSA on crosslinked DEAE dextran has been determined experimentally. They were little affected by the initial concentration of BSA but were considerably affected by pH, ionic strength, and temperature. Adsorption isotherms were correlated by the Langmuir isotherm, Freundlich isotherm, and Freundlich–Langmuir isotherm.[10] Adsorption capacity factor and adsorption equilibrium constant were found. Mathematical modeling of adsorption and kinetic order, adsorption rate constant, and maximum adsorption was performed for two systems. Using the fitting results, we extracted some ideas for the adsorption mechanism of a protein on crosslinked DEAE Dextran. It has been proved experimentally and theoretically that protein is adsorbed by electrostatic attraction, ion-exchange, hydrophobic interaction, and/or hydrogen bonding. Swelling kinetics of crosslinked DEAE dextran and optimum ionic strength, pH, and mass of hydrogel were investigated. Desorption studies were determined at optimum medium conditions of DEAE dextran microbeads and protein systems. Adsorption thermodynamics of BSA has been studied on the microbeads on N,N′-Dimethylaminoethyl (DEAE) dextrananion exchanger at different temperatures.[11]

In this paper, we investigate the effect of temperature on the HSA adsorption onto DEAE dextran microbeads. Thermodynamic parameters of the adsorption of HSA on weakly anion exchanger DEAE dextran microbeads was determined by experimentally and general thermodynamic aspects have been discussed. Association free energy (ΔG_{assoc}) and entropy change of the adsorption (ΔS_{assoc}) were evaluated at different temperatures. The association heat of the adsorption (ΔH_{assoc}) involved in the molecule transfer from the mobile to the stationary phase was calculated by using the linear Van't Hoff plot.



Scheme 1 Schematic representation of *N,N′*-diethylaminoethyl (DEAE) dextran.

EXPERIMENTAL

Materials

Commercial diethylaminoethyl (DEAE) dextran microbeads were used. (Sephadex A-50, Pharmacia Fine Chemicals) (Scheme 1)

Human serum albumin (HSA) was purchased from Sigma (Lyophilized, fraction V). Adsorption studies were performed in HSA reservoir with constant stirring at rate at 120 rev/min. Some properties of the crosslinked DEAE dextran microbeads are shown in the Table I.[7]

Adsorption studies

Adsorption experiments were carried out at five different temperatures of 25, 30, 35, 40, and 45°C. Microbeads were contacted with the HSA solution and gently mixed. The amount of HSA adsorbed on the particles was measured after a certain time and until it was confirmed that there was no further adsorption. The adsorption capacity was determined by measuring the initial and final concentrations of HSA within the reservoir, using a Schimadzu-100 double beam UV–vis spectrophotometer at $\lambda = 280$ nm. Using the formula the adsorbed phase concentration of HSA was calculated with;

$$q_{eq} = V(c_0 - c_{eq})/W \quad (1)$$

where c_0 and c_{eq} are the initial and equilibrium concentrations of HSA in liquid phase (mg/mL), respectively, q_{eq} denotes the microbeads-phase concentration of HSA (mg HSA/g of crosslinked DEAE dextran microbeads) and W is the mass of the microbeads (g) and V is the volume of the solution (mL).

TABLE I
Properties of Crosslinked Diethylaminoethyl (DEAE) Dextran Sephadex (A-50 Ion-Exchanger)[7]

Description	Weakly basic anion exchanger
Functional group	Diethylaminoethyl (DEAF)
Diameter in water[a] (mm)	
Free	0.2364
Saturated by BSA	0.2182
Diameter of dry particle (mm)	0.0851
Water content (% wt)	94.1
Effective pH range	2–9
pK of crosslinked DEAE dextran group	9.5
Density	
True (kg/m^3)	1690
Apparent (kg/m^3)	604
Porosity	0.963
Total capacity	3.5 ± 0.5 mg/g
Available capacity	
Albumin (*MW* = 67,000)	7566 mg/g
Hemoglobin (*MW* = 69,000)	5000 mg/g
Ferritin (*MW* = 440,000)	74 mg/g

[a] Average value of 50 particles.

RESULTS

Effect of temperature on adsorption of HSA onto crosslinked DEAE dextran microbeads

The equilibrium studies on the crosslinked DEAE dextran ion-exchangers have been of great interest because of their wide range of applications. A crucial aspect of protein/surface phenomena is the effect of temperature on the protein adsorption and protein structure, and consequently, on the biological activity of the protein.[12] The interactions of protein with charged hydrophilic surfaces (e.g., ion-exchanger adsorbents) have not been well characterized on a fundamental level. There is extensive body of literature concerning the use of hydrophilic polyelectrolyte surfaces in the chromatographic separation of proteins, but equilibrium studies of the thermodynamics of protein adsorption on chromatographic adsorbents are uncommon. The resolution with which variations in thermodynamic behavior as a function of protein loading and temperature can be detected by batch equilibrium experiments and indirect method Van't Hoff plot analysis were used.[13]

The adsorption isotherms of adsorption of HSA on crosslinked DEAE dextran microbeads have been investigated by varying the temperature in the range of 25–45°C and follow adequately a Freundlich adsorption behavior[14] and can be represented by the equation:

$$q_{eq} = K_D c_{eq}^{1/n} \quad (2)$$

$$\text{In } q_{eq} = In\ K_D \times I/n\ In c_{eq} \quad (3)$$

where q_{eq} is the equilibrium concentration of the solute in the solution in mg/L. q_{eq} is the amount of solute adsorbed onto the unit mass of the adsorbent (mg/L) for certain period of time, respectively. The slope and intercept of the linear Freundlich equation are equal to $1/n$ and ln K_D, respectively. K_D and n are empirical constants dependent on the rate of solid and adsorbate and on the temperature. According to the Freundlich equation, the amount adsorbed increases infinitely with increasing concentration.[14]

Experimental equilibrium binding data were generated at different temperatures, the corresponding adsorption isotherms were constructed, and equilibrium parameters (q_{eq} and K_D) were determined from the corresponding semireciprocal plots fitted to the points by the least squares method (Figs. 1 and 2).[14] Obtained association constant values are given in the Table II depending on the temperature. The observed decrease in K_D values with the temperature may be due to the following factors. At higher temperature during the unfolding process, the proteins expose buried amino acid residues on the surface. Thus the contact area between the protein and the functional groups of the matrix should increase, resulting in an increase in the binding sites of the protein for the adsorbent at higher temperatures.

The equilibrium association constant, (K_D) extracted from the semireciprocal plot was then employed for the Van't Hoff plot analysis of log K_D versus the reciprocal of the temperature. From the Van't Hoff plot, the apparent thermodynamic parameters (ΔG_{assoc}, ΔH_{assoc}, and ΔS_{assoc}) were then extracted. It should be noted that the thermodynamic parameters extracted are apparent values, since the phase ratio, i.e., the ratio of the volume of the stationary phase to that of mobile phase in the finite batch is not specified at the different temper-

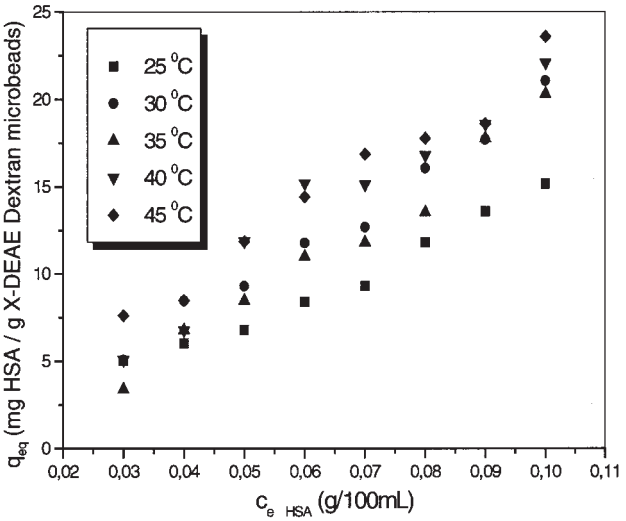


Figure 1 Temperature dependence of adsorption capacity of the crosslinked DEAE dextran/HSA system (semireciprocal plots of the experimental data for the adsorption isotherm of HSA on crosslinked DEAE dextran microbeads at different temperatures).

atures, however, for the purpose of these analyses, it was assumed to be constant.

The value of the apparent change in enthalpy (ΔH_{assoc}) during the binding process was determined from the gradient of the plots and can be equated inter alia with the extent of hydrogen bond formation or breakage. The corresponding values of the apparent change in free energy (ΔG_{assoc}) and the apparent change in entropy (ΔS_{assoc}) were determined from the relationships

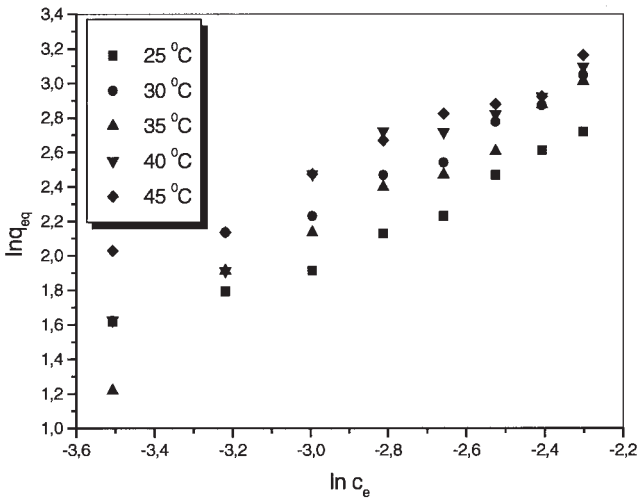


Figure 2 Linear form of the temperature-dependence of adsorption capacity of the crosslinked DEAE dextran/Human serum albumin system (semi-reciprocal plots of the experimental data for the adsorption isotherm of HSA on crosslinked DEAE dextran microbeads at different temperatures).

TABLE II
Values of Association Equilibrium Constant K_D (or Thermodynamic Coefficient) for the Adsorption of HSA onto Crosslinked DEAE Dextran Microbeads at Different Temperatures

Temperature (K)	K_D
298	122.7
303	252.1
308	464.1
313	365.0
318	194.4

$$\Delta G_{assoc} = -RT\ln K_D \quad (4)$$

$$\Delta G_{assoc} = \Delta H_{assoc} - T\Delta S_{assoc} \quad (5)$$

where R is the gas constant. The effect of temperature in polymer/protein interaction may also be important in such a system.

The results, depicted in Figures 1, 2, and Table II imply that with increasing temperature, HSA adsorption decreases.

(i) With increasing temperature, the electrostatic binding forces between the HSA molecules and polymer surfaces weaken, and adsorption decreases.
(ii) At higher temperatures, the escaping tendency of the HSA molecules from the surface to the bulk solution increases, which also result in decreased adsorption.
(iii) At lower temperatures, the protein molecules may acquire a more compact structure, which will result in great adsorption.

One can not also rule out the possibility of physical agglomeration of the HSA molecule at lower temperature (35°C), which also results in increased adsorption. Such agglomeration has also been postulated by several workers in the case of dye adsorption.[15–17]

Thermodynamic parameters of the adsorption of HSA onto crosslinked DEAE dextran microbeads

The dependence of the equilibrium association constant, K_D, on $1/T$ for the binding of human serum albumin on DEAE dextran microbeads was analyzed in terms of Van't Hoff plots. The Van't Hoff plot was linear for the system (Fig. 3). This linear behavior was thermodynamically what was expected where there was no change in the interaction mechanism in relation to temperature.

The ΔG_{assoc} values for HSA adsorbed on the crosslinked DEAE dextran microbeads were calculated for each temperature and tabulated in Table III. The negative ΔG_{assoc} values for each temperature in-

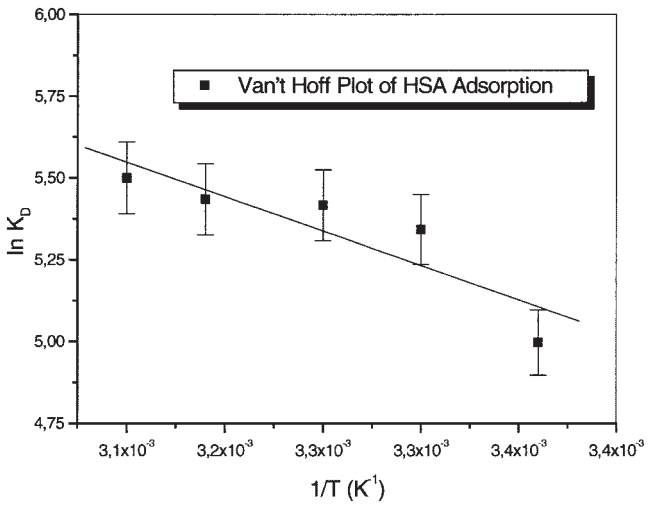


Figure 3 Van't Hoff plot for the adsorption of human serum albumin adsorbed onto crosslinked DEAE dextran microbeads.

dicated that adsorption of HSA on the crosslinked DEAE dextran was a favorable process and those were ranged between −12.380 and −14.541 kJ/mol. The high affinity of the adsorption isotherm even at significant loadings implies that ΔG_{assoc} is negative under the conditions used. The homogeneous nature of the adsorbent surface and ill-characterized surface–charge density and distribution, however, preclude calculation of a meaningful value of ΔG_{assoc}. Furthermore, the Van't Hoff analysis of ten used for calculation of ΔH_{assoc} is based on the assumption that a reversible equilibrium exists between the bound and the free protein. It lumps together all subprocesses accompanying protein adsorption and is therefore only a qualitative indicator of adsorption thermodynamics[13] ΔS_{assoc} values for the adsorption of HSA to crosslinked DEAE dextran also are presented in Table III. At lower temperatures, due to lower kinetic motion of the HSA, the value of the phase ratio will be different from to that at higher temperatures. Positive values for the ΔS_{assoc} were obtained for the adsorption of HSA onto crosslinked DEAE dextran microbeads, indicating an increase in the total disorder of the system during adsorption. The apparent Van't Hoff enthalpy ΔH_{assoc} of complex formation was determined from the temperature dependencies of the equilibrium constants (Fig. 3). At lower temperatures, because of lower kinetic motion of the HSA, the value of the phase ratio will be different to that at higher temperatures. Positive values for the ΔS_{assoc} were obtained for the adsorption of HSA onto crosslinked DEAE dextran microbeads, indicating an increase in the total disorder of the system during adsorption. The apparent Van't Hoff enthalpy, ΔH_{assoc} of complex formation was determined from the temperature dependencies of the equilibrium constants (Fig. 3).

The calculated ΔH_{assoc} value of the system for the interaction for HSA adsorbed onto crosslinked DEAE dextran microbeads was 20.650 kJ/mol. Such determinations assume that ΔH_{assoc} is independent of temperature, and that consequently, there is no change in the heat capacity of the system. The good linearity of the Van't Hoff plots strongly suggests that it is indeed the case, at least in the temperature domain, where our experiments were performed (25 and 45°C). Obtained values indicate that complexation reaction with HSA and crosslinked DEAE dextran microbeads is entropically driven and accompanied by an unfavorable enthalpy variation since the values of ΔH_{assoc} is positive.[18–20]

The interactions between ionic species in aqueous solution are characterized by small positive enthalpy and positive entropy changes. Accordingly, the present thermodynamic behavior corresponded to the model describing the electrostatic attraction that occurs between the negatively charged nonspecific regions of HSA and the positively charged crosslinked DEAE dextran microspheres. These results approved that adsorption isotherm, ΔH_{assoc} value of the, all the spectroscopic methods for the adsorption and desorption are quite harmonious. The amount of energy required for association of adsorption is 20.7 kJ/mol and value of this energy is an evidence of the strong binding nature of the adsorption.

DISCUSSION

Accordingly, the present thermodynamic behavior corresponded to the model describing the electrostatic attraction that occurs between the negatively charged nonspecific regions of HSA and the positively charged crosslinked DEAE dextran microspheres. These results approved that adsorption isotherm, ΔH_{assoc} value of the, all the parameters for the adsorption are quite harmonious. The amount of energy required for association of adsorption is 20.7 kJ/mol and the value of this energy is evidence of the strong binding nature of the adsorption.

TABLE III
Change in the Free Energy (ΔG_{assoc}) and Entropy (ΔS_{assoc}) for the Adsorption of Human Serum Albumin (HSA) onto Crosslinked DEAE Dextran Microbeads at Different Temperatures

Temperature (K)	ΔG_{assoc} (kJ/moI)	ΔS_{assoc} (J/mol K)
298	−12.380	110.84
303	−13.457	112.57
308	−13.869	112.07
313	−11.141	111.15
318	−14.541	110.06

Concerning the specific binding, our results of the HSA/dextran show that the thermodynamic parameters are fairly different from those previously obtained with the BSA/dextran system.[10,11] The values obtained for ΔH_{assoc} (20.7 kJ/mol) and ΔS_{assoc} (112 J/(mol °C)) are more reminiscent of the specific binding of the HSA, showing a large entropic favorable contribution and a positive enthalpic term. As for the specific binding it is therefore probable that the increase in binding energy is related to supplementary hydrophobic contacts. There appears to be many factors involved in desorption curves not always following the adsorption curves. Firstly, under conditions where interfacial coagulation occurs, a true adsorption isotherm is not obtained. Secondly, desorption under certain conditions may be an extremely slow process, whereas, the adsorption of a protein molecule to enter the interface, desorption by necessity requires that a large number of segments leave the interface together. Each of these segments may have an appreciably high free adsorption energy and due to the high free energy of the segments, the total free energy of the molecule may become high. These results support the interpretation that the HSA has a moderate affinity for crosslinked DEAE dextran. At the same time when discussing the enthalpy of the HSA adsorption process, the good linearity of the Van't Hoff plots strongly suggest that it is indeed ΔH_{assoc} value found for the specific binding.

This study is carried out Department of Chemistry of Hacettepe University.

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