A Comparative Study of the Interaction between Nafcillin and Catalase by Equilibrium Dialysis and ζ-Potential Measurements

ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · APRIL 2001

Impact Factor: 3.3 · DOI: 10.1021/jp003404m

5 AUTHORS, INCLUDING:

Juan M. Ruso
University of Santiago de Compostela
163 PUBLICATIONS **2,005** CITATIONS
SEE PROFILE

Pablo Taboada
University of Santiago de Compostela
147 PUBLICATIONS **2,270** CITATIONS
SEE PROFILE

Xerardo Prieto
University of Santiago de Compostela
111 PUBLICATIONS **1,659** CITATIONS
SEE PROFILE

Félix Sarmiento
University of Santiago de Compostela
158 PUBLICATIONS **2,453** CITATIONS
SEE PROFILE

A Comparative Study of the Interaction between Nafcillin and Catalase by Equilibrium Dialysis and ζ-Potential Measurements

Juan M. Ruso, Pablo Taboada, Pablo Martínez-Landeira, Gerardo Prieto, and Félix Sarmiento*

Grupo de Física de Coloides y Polímeros, Departamento de Física Aplicada y Departamento de Física de la Materia Condensada, Facultad de Física, Universidad de Santiago de Compostela, 15706 Santiago de Compostela, Spain

Received: September 21, 2000; In Final Form: January 19, 2001

The characterization of the interactions of the penicillin nafcillin with the protein catalase in aqueous solution at pH 3.2, 7.4, and 10.0 at 25 °C has been determined using a combination of equilibrium dialysis and ζ-potential measurements. At pH 3.2, the ζ-potential of catalase tends to reverse charge from positive to negative values as the negatively charged penicillin bind to it. At pH 7.4 and 10.0, this ζ-potential becomes more negative as the nafcillin concentration increases. The number of drug molecules bound to catalase per protein molecule (υ) was obtained from equilibrium dialysis and compared with the ζ-potential data. Nafcillin binds largely to catalase to various extents ranging from ∼750 (pH 3.2) to ∼10 000 (pH 7.4 and 10.0) drug molecules per catalase molecule as the free concentration approaches the critical micelle concentration (cmc) of the free drug. Gibbs energies per drug molecule bound ($\Delta G_{\bar{\upsilon}}$) were obtained as a function of $\bar{\upsilon}$ from equilibrium dialysis using the Wyman potential (*J. Mol. Biol.* **1965**, *11*, 631). $\Delta G_{\bar{\upsilon}}$ is large and negative at low values of penicillin concentration and becomes less negative as the drug concentration increases. $\Delta G_{\bar{\upsilon}}$ against drug concentration plots obtained from the interpretation of the variation of ζ-potential measurements were of the form and magnitude similar to those obtained from direct binding measurements using the equilibrium dialysis technique.

1. Introduction

Folding and binding are two of the most fundamental aspects of protein behavior. Biological function is generally possible only when a protein is folded into a specific three-dimensional conformation; unfolded proteins are impotent. Biological function also involves the interaction with other molecules: enzymes bind their substrates and products, and transport proteins bind their ligands. Folding involves the interaction of two initially flexible surfaces, whereas in binding, generally only the ligand is substantially flexible. The interaction of small molecules with macromolecules of biological systems and with specific receptor sites on surfaces of supramolecular organizations is one of the most extensively studied phenomena in biophysical research. The binding subjects include a vast range of important biochemical phenomena: the reversible binding of oxygen by mioglobins and the noncovalent association of serum albumin with fatty acid anions and other compounds containing nonpolar groups.[1,2] Also, the interaction of amphiphilic molecules with proteins or enzymes has been subject of numerous investigations over several decades.[3−5] Amphiphilic molecules are believed to bind initially to specific opposite charged sites, the subsequent binding being largely hydrophobic and nonspecific.

In previous reports, we have studied the interactions between different proteins and amphiphilic ligands (insulin-*n*-alkyl sulfates,[6−8] insulin-*n*-alkyltrimethylammoniumbromides[9,] insulin-imipramine,[10] lysozyme-*n*-alkyltrimethylammoniumbromides,[11,12] and lysozyme-*n*-alkyl sulfates[13]) using as physical methods equilibrium dialysis, difference spectroscopy, microcalorimetry, and ζ-potential. The results have permitted us to obtain a thermodynamic picture of the nature of the protein−amphiphilic interaction. Recently, we have reported an investigation on the interaction of a range of synthetic penicillins with human serum albumin (HSA).[14,15] The penicillins nafcillin, cloxacillin, dicloxacillin, and flucloxacillin were found to bind extensively to HSA in aqueous solution. The binding is exothermic and dominated by large increases in entropy, characteristics that are similar to the interactions of anionic surfactants with globular proteins.[5] The strongest interaction was found for nafcillin, which suggests a greater hydrophobic contribution arising from the naphthalene ring than the monohalide interactions. The application of ζ-potential measurements to the study of these penicillins with HSA gives Gibbs energies of interaction as a function of the number of drug molecules bound that are of similar shape and magnitude to those in data obtained by direct measurements of the extents of drug binding.

The hydrophobic nature of nafcillin, as demostrated by their self-association behavior,[16] and the strong character of the HSA−nafcillin interaction suggest that noncovalent binding of nafcillin is an important property of the penicillin requiring more detailed study. For this reason, we have chosen a more complex protein: catalase. Catalase (hydrogen peroxide: hydrogen peroxide−oxidoreductase, EC 1.11:1.6), present in the perixisomes of nearly all aerobic cells, serves to protect the cell from the toxic effects of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water without the production of free radicals. The protein exists as a dumbbell-shaped tetramer of four identical subunits, each subunit formed by a single polypeptide chain with haemin as a prosthetic group.[17,18] Catalase was one of the first enzymes to be purified to

* Corresponding author. Tel.: +34 981 563 100. Fax: +34 981 520 676. E-mail: fsarmi@usc.es.

10.1021/jp003404m CCC: $20.00
Published on Web 03/08/2001

homogeneity and has been the subject of intense study by several physical methods.[19−25] As added scientific value, the knowledge of the catalase−nafcillin interactions should help to increase our understanding of the mechanism of action of catalase, which is at present subject of a wide study.

2. Experimental Section

Materials. Bovine liver catalase (Product No. 9322) and nafcillin (6-2-[ethoxy-L-naphthamidol]-penicillin, sodium salt (Product No. D-3269) were obtained from Sigma Chemical Co. Three buffers were used: (1) glycine (50×10^{-3} mol dm^{-3}) plus hydrochloric acid pH 3.2, (2) NaH_2PO_4 (0.2 mol dm^{-3}) plus Na_2HPO_4 pH 7.4, and (3) glycine (50×10^{-3} mol dm^{-3}) plus sodium hidroxyde, pH 10. Sodium azide (0.02% w/v) obtained from Merck (No. 6688) was added to the three buffers, contributing 0.0031 mol dm^{-3} to the ionic strength. All other materials were of analytical grade, and solutions were made using doubly distilled and degassed water.

Adsorption of Nafcillin by Catalase. To obtain a complete adsorption onto the protein, we placed aliquots of 2.5 cm^3 of the protein solution of concentration 0.125% w/v in a test tube and equilibrated them with 2.5 cm^3 of nafcillin solutions for over 3 h, covering the required range of concentration.

Zeta Potential Measurements. The ζ-potentials of the systems previously exposed were measured using a Malvern Instruments Ltd. Zetamaster 5002 by taking the average of five measurements at stationary level. The cell used was a 5 mm × 2 mm rectangular quartz capillary. The temperature of the experiments was 25.0 °C controlled by a Heto proportional temperature controller.

The ionic strength of the buffers was 0.0119, 0.0176, and 0.0318 for pH 3.2, 7.4, and 10.0, respectively. So the Debye−Hückel parameters (κ) were 0.899, 1.33, and 2.40 for the three pH's under study. If a protein radius (a) of approximately 10 nm was assumed, estimated from crystallographic studies,[26] the products κa were 8.99, 13.3, and 24.0, corresponding to the Henry factors $f(\kappa a)$ of 1.109, 1.243, and 1.350[27] for 3.2, 7.4, and 10.0 pH, respectively. The ζ-potentials were calculated from the electrophoretic mobilities (u_E), using the equation

$$\zeta = \frac{3u_E\eta}{2\epsilon_0\epsilon_r}\frac{1}{f(\kappa a)} \qquad (1)$$

where the permittivity of vacuum (ϵ_0), relative permittivity (ϵ_r), and viscosity of water (η) were taken as 8.854×10^{-12} J^{-1} C^2 m^{-1} and 78.5 and 8.904×10^{-4} N m^{-2} s, respectively.

Titration. To obtain measurements of ζ-potential at different pHs, we connected the zetamaster to a Mettler DL21 Titrator, which is a complete analysis station for titrametric analyses.

Equilibrium Dialysis. Dialysis is based on differences in molecular size. In dialysis, two compartments are separated by a semipermeable membrane being (ideally) perfectly permeable to one interactant, say, the ligand (nafcillin), and perfectly impermeable to the substrate (catalase). After equilibrium is achieved, the two compartments are analyzed for their total concentrations of ligand. Since the activity of free (unbound) ligand is identical in the two compartments at equilibrium, the analytical data yield the concentration of free ligand and, by difference, the concentration of bound ligand in the compartment containing the substrate. Aliquots (2 cm^3) of catalase (concentration 0.125% w/v at the three pHs under study) in dialysis bags were equilibrated with 5 cm^3 of nafcillin solution for each buffer, covering the range of concentrations in screw cap sample tubes held in a thermostated bath at 25 °C for a period of 96 h.

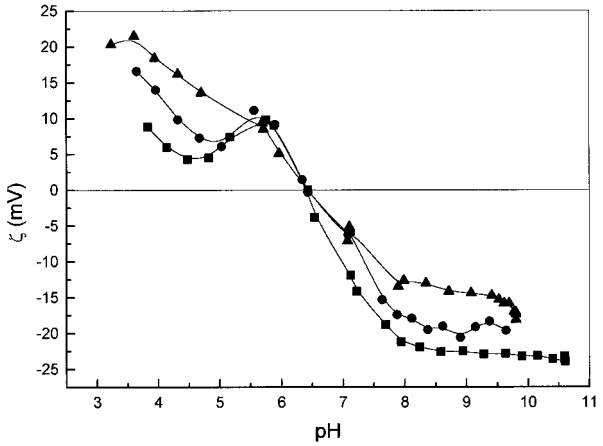

Figure 1. Titration curves for catalase (initial concentration 0.125% w/v) in the presence of different glycine concentrations: (■) 15×10^{-3} mol kg^{-1}, (●) 50×10^{-3} mol kg^{-1}, and (▲) 100×10^{-3} mol kg^{-1}.

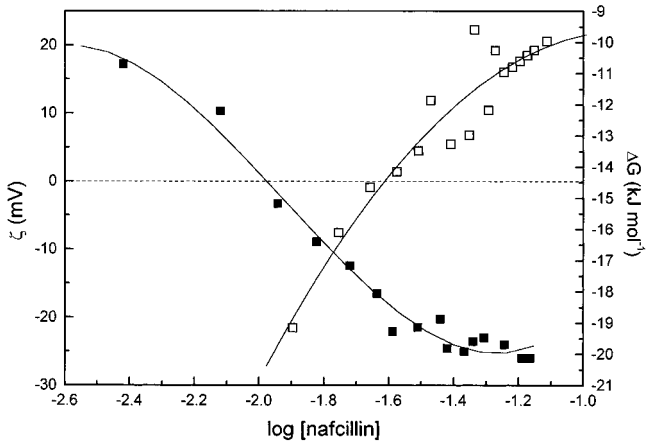

Figure 2. ζ-Potential (■) and Gibbs energies of the adsorption (□) of bovine catalase (0.125% w/v) in glycine buffer (ionic strength 0.0119 M, pH 3.2, 25 °C) as a function of logarithm of nafcillin concentration.

Spectroscopy. Difference spectra were measured using a Beckman spectrophotometer (model DU 640), with six microcuvettes operating in the UV−visible region and a full-scale expansion of 0.2 absorbance units. For absorbance difference spectra, five of the six microcuvettes were filled with protein and surfactant solutions. The first microcuvette contained only protein in the corresponding medium and was used as a blank reference. The microcuvettes were filled and placed in the same orientation for all the tests. Absorbance was measured at temperature of 25 °C using a temperature controller (Beckman DU Series) on the basis of the Peltier effect.

3. Results and Discussion

Figure 1 shows the ζ-potentials of catalase as a function of pH at various concentrations of glycine (15, 50, and 100×10^{-3} mol kg^{-1}). The isoelectric point is determined by the pH at which the ζ-potential becomes zero and corresponds to a value of 6.4 in these experiments. The fact that it is independent of the ionic strength of the glycine suggests that this is an indifferent electrolyte in this system, as could be expected.

The zeta potential of catalase in the presence of nafcillin for the three pH's 3.2, 7.4, and 10.0 is shown in Figures 2−4, respectively. At pH 3.2, where the net charge of catalase is positive (see Figure 1), the same sign is observed at low drug concentrations. The adsorption of nafcillin onto catalase tends to reverse the sign of the ζ-potential. For this pH, no higher

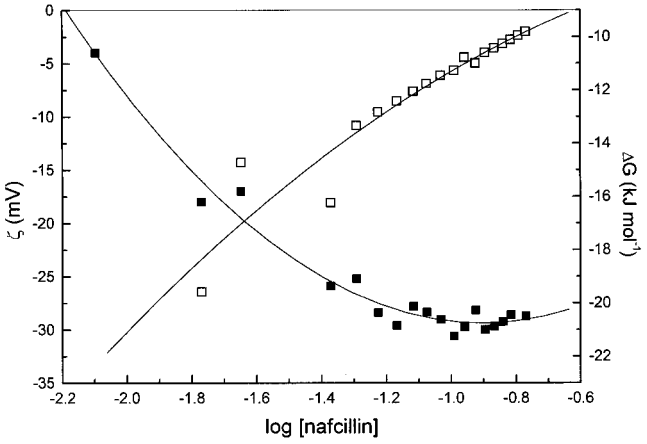

Figure 3. ζ-Potential (■) and Gibbs energies of adsorption (□) of bovine catalase (0.125% w/v) in phosphate buffer (ionic strength 0.0176 M, pH 7.4, 25 °C) as a function of logarithm of nafcillin concentration.

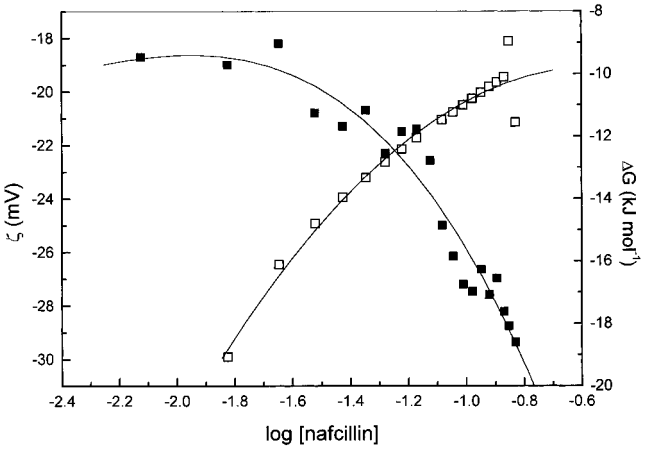

Figure 4. ζ-Potential (■) and Gibbs energies of the adsorption (□) of bovine catalase (0.125% w/v) in glycine buffer (ionic strength 0.0318 M, pH 10.0, 25 °C) as a function of logarithm of nafcillin concentration.

nafcillin concentrations were used due to the precipitation of the drug. For pH 7.4 and 10.0, all the negative ζ-potential values become more negative as the drug concentration increases. Electrostatic interactions between the headgroup of the drug (negative) and the oppositely charged amino acid residues (positives) occur chiefly at low drug concentrations at pH 3.2. For the other pH and negative values of the ζ-potential at pH 3.2, hydrophobic interactions are predominant, arising from the fact of the hydrophobic nature of the penicillin and the presence of hydrophobic heme groups, which are deeply embedded in each subunit of the protein structure.[26,28,29] Although the net charge of the protein is the same for pH 7.4 and 10 and the interactions are mainly hydrophobic, for pH 7.4, there is an initial decrease of the ζ-potential, which tends to a final plateau. However, at pH 10.0, the plateau is the initial response to drug addition, and a sudden decrease is observed. Previous studies have shown how the tertiary structure of catalase is modified by SDS in the monomeric and micellar form and how the secondary structure is altered only in the presence of SDS micelles.[30] A drastic change in the catalase structure at pH 10.0 at some nafcillin concentration, allowing a great adsorption of nafcillin molecules onto the protein, could be the more reasonable explication.

Considering the slope of the ζ-potential-log nafcillin concentration as adsorption due to the hydrophobic effect taking place (i.e. the whole of concentration under study, where the protein and the monomer have the same charge sign), we can

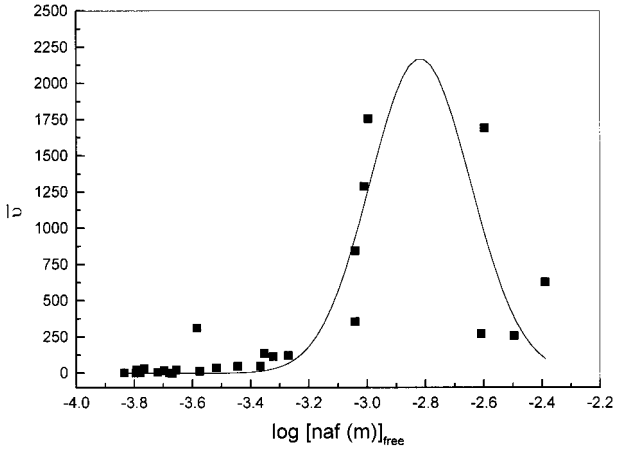

Figure 5. Binding isotherm for nafcillin on bovine catalase in aqueous solution at pH 3.2. $\bar{\upsilon}$ is the number of nafcillin molecules bound per protein molecule.

use the following equation to calculate the number of adsorption sites, N_1:[31, 32]

$$\left(\frac{\mathrm{d}}{\mathrm{d}\log c}\right)=\frac{4.606k_{\mathrm{B}}T}{ze}\left(\frac{\sinh(ze\zeta_1/2k_{\mathrm{B}}T)-\sinh(ze\zeta_2/2k_{\mathrm{B}}T)}{\cosh(ze\zeta_2/2k_{\mathrm{B}}T)}\right)\times\left(\frac{\sqrt{8n_0\epsilon k_{\mathrm{B}}T}[\sinh(ze\zeta_1/2k_{\mathrm{B}}T)-\sinh(ze\zeta_2/2k_{\mathrm{B}}T)]}{zeN_1}-1\right) \quad (2)$$

where ζ_1 and ζ_2 are the ζ-potentials on the line, k_B is the Boltzmann constant, c the concentration, T the absolute temperature, n_0 the ionic concentration, e the proton charge, z the valence of the ion, and ϵ the relative permittivity of the medium.

The adsorption constant k_2 can be calculated from the equation

$$\frac{1}{c}=k_2\left(\frac{zeN_1}{\sqrt{8n_0\epsilon k_{\mathrm{B}}T}[\sinh(ze\zeta_1/2k_{\mathrm{B}}T)-\sinh(ze\zeta_2/2k_{\mathrm{B}}T)]}-1\right) \quad (3)$$

Here, c is chosen as the concentration at the ζ-potential midpoint between ζ_1 and ζ_2. The standard free energy of adsorption, ΔG^0_{ads}, can be obtained from the equation

$$k_2=\exp(-\Delta G^0_{ads}/k_{\mathrm{B}}T) \quad (4)$$

The standard Gibbs energies of adsorption evaluated from eq 4 are plotted in Figures 2 to 4. The results show that the Gibbs energies are large and negative at low penicillin concentrations, where binding to the high energy sites takes place, and become less negative as more nafcillin molecules bind. Similar behavior was found for different systems: sodium n-dodecyl plus sulfate histone HI,[33] sodium n-dodecyl sulfate plus lysozyme,[34] and nafcillin plus human serum albumin.[14]

The adsorption isotherms for the nafcillin on catalase plotted as the number of drug molecules per protein molecule, $\bar{\upsilon}$, as a function of the log (free drug concentration) are shown in Figures 5−7. At pH 3.2, this kind of isotherm is characteristic of at least two sets of binding sites; initially, binding of the anionic nafcillin to cationic residues occurs, followed by hydrophobic binding at the drug concentration approaching the cmc (which was taken from a previous study[16] and is indicate by an arrow in the figures). The observation is coherent with the observed change of ζ-potential with concentration (Figure 1). The isotherm passes through maxima which have been previously observed in the adsorption isotherms of surfactant binding to globular proteins[35] and insoluble polymeric sub-

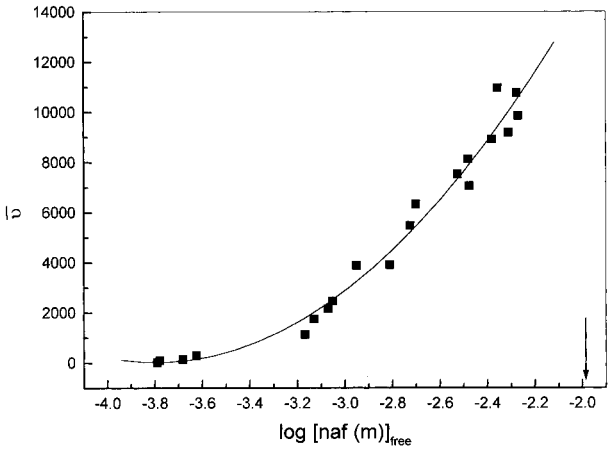

Figure 6. Binding isotherm for nafcillin on bovine catalase in aqueous solution at pH 7.4. The cmc of nafcillin is indicated with an arrow. $\bar{\upsilon}$ is the number of nafcillin molecules bound per protein molecule.

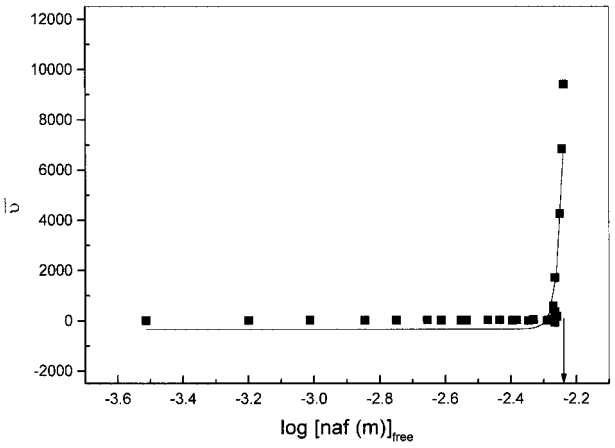

Figure 7. Binding isotherm for nafcillin on bovine catalase in aqueous solution at pH 10.0. The cmc of nafcillin is indicated with an arrow. $\bar{\upsilon}$ is the number of nafcillin molecules bound per protein molecule.

strates[36,37] and penicillins cloxacillin and nafcillin binding to human serum albumin.[14] This behavior has been attributed to the activity of long-chain ions going through a maximum in the vicinity of the cmc.[38,39] At pH 7.4 and 10.0, specific binding to cationic sites is lost, and only hydrophobic binding occurs. The binding isotherms for the nafcillin range up to $\bar{\upsilon}$ values of 755, 10 954, and 9397 for 3.2, 7.4, and 10.0, respectively. Catalase has four subunits of equal molecular weight, each subunit consisting of a 506 amino acid polypeptide chain plus one heme group and one NADH molecule,[40] so the number of drug molecules per amino acid residue is 0.37, 5.4, and 4.64 for pH 3.2, 7.4, and 10.0, respectively. We must mention that for pH 3.2, low nafcillin concentrations were used due to the precipitation of the drug, explaining the low number of drug molecules per amino acid. For the other systems (at different pHs), the average number obtained is similar and very close to the value obtained for the system nafcillin human serum albumin, which was 5,[14] the same value corresponding to the aggregation number of the drug micelle.[16]

The Gibbs energies of binding per mole of drug ($\Delta G_{\bar{\upsilon}}$) were calculated from the Wyman binding potential (Π) derived from the area under the binding isotherms according to the equation[41]

$$\Pi = RT\int_0^{\bar{\upsilon}} \bar{\upsilon}\, \mathrm{d}\ln[S] \qquad (5)$$

where R is the gas constant, T the absolute temperature, and

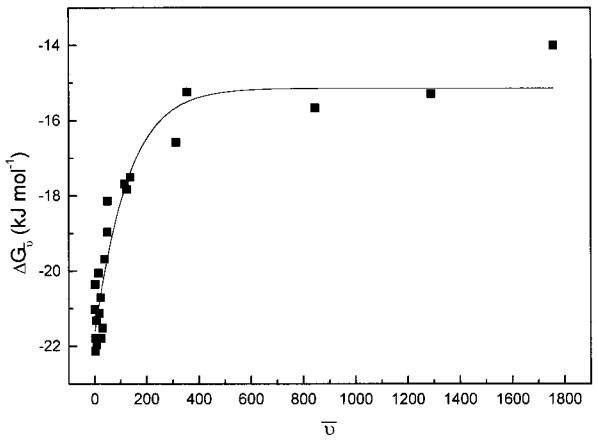

Figure 8. Gibbs energies of interaction per mol of bovine catalase as a function of the number of nafcillin molecules bound to catalase molecule ($\bar{\upsilon}$) at pH 3.2, ionic strength 0.0119 M, and 25 °C

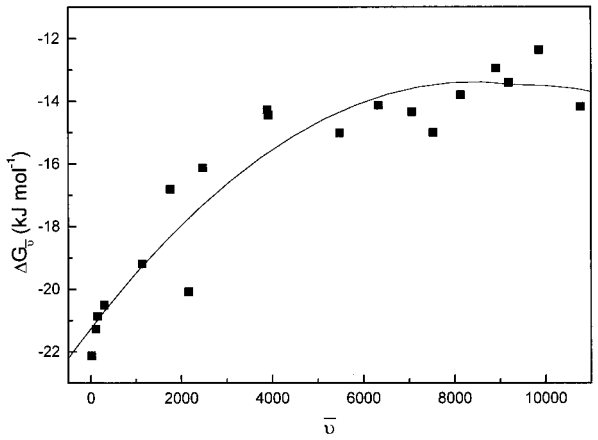

Figure 9. Gibbs energies of interaction per mol of bovine catalase as a function of the number of nafcillin molecules bound to catalase molecule ($\bar{\upsilon}$) at pH 7.4, ionic strength 0.0176 M, and 25 °C.

[S] the free drug concentration. The binding potential is related to the apparent binding constant, K_{app}, as follows

$$\Pi = RT\ln(1 + K_{app}[S]^{\bar{\upsilon}}) \qquad (6)$$

The Gibbs energy of binding is calculated from

$$\Delta G_{\bar{\upsilon}} = -\frac{RT}{\bar{\upsilon}}\ln K_{app} \qquad (7)$$

Figures 8−10 show $\Delta G_{\bar{\upsilon}}$ as a function of $\bar{\upsilon}$ for the pHs under study. The shape of these curves shows that $\Delta G_{\bar{\upsilon}}$ is large and negative at low values of $\bar{\upsilon}$, where binding to the high-energy sites occurs and gradually decreases as saturation is approached. To gain quantitative understanding of the influence of pH on the strength of binding, the numerical values of $\Delta G_{\bar{\upsilon}}$ at $\bar{\upsilon} \approx 150$ were calculated. The results obtained were −18 kJ mol^{-1} at pH 3.2, −21 kJ mol^{-1} at pH 7.4, and −14 kJ mol^{-1} at pH 10.0. These values are lower than those obtained for the adsorption of SDS onto catalase[23] but similar to those calculated for the human serum albumin−nafcillin system.[14] The electrical contributions to the Gibbs energies of binding can be approximately estimated from the expression $\pm z_i e\zeta_i$, where z_i is the charge on the adsorbing ion (here, −1), e is the electronic charge per mole, and ζ_i the ζ-potential.[42] From the ζ-potentials (Figures 2, 3 and 4) and binding data (Figures 5, 6 and 7), the electrical contributions at $\bar{\upsilon} \approx 150$ were −17 kJ mol^{-1} at pH 3.2, −17 kJ mol^{-1} at pH 7.4, and −10 kJ mol^{-1} at pH 10.0.

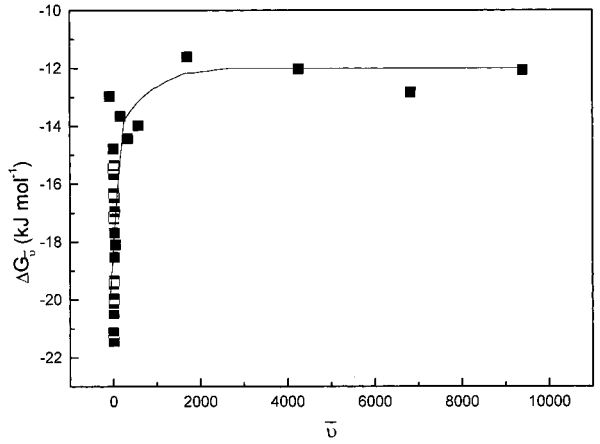

Figure 10. Gibbs energies of interaction per mol of bovine catalase as a function of the number of nafcillin molecules bound to catalase molecule ($\bar{\upsilon}$) at pH 10.0, ionic strength 0.0318 M, and 25 °C.

These electrical contributions are 94% (pH 3.2), 81% (pH 7.4), and 71% (pH 10.0) of the total Gibbs energies of binding. In summary, equilibrium dialysis and ζ-potential are presented here to achieve a better and broader understanding in the study of drug−protein interactions. An increase in the number of adsorbed molecules of nafcillin onto catalase is well correlated with increments in the net charge of the nafcillin−catalase complex calculated by ζ-potential measurements. The sudden rise in $\bar{\upsilon}$ found for pH 10.0 can be observed in the final decrease of ζ-potential shown for the same system. Results obtained from the free energies of adsorption by both methods are on the same order, but those obtained from ζ-potential measurements are always lower. Although both techniques are simple in practice, there are some limitations. Equilibrium dialysis is valid to state that the chemical potential of unbound ligand is identical in the two compartments and, therefore, that the activities of unbound ligand are the same, since we reasonably choose the same standard state for ligand in each compartment. But it does not necessarily follow that the concentrations of ligand are identical because it is conceivable that the ligand activity coefficients are different in the two compartments, whose contents obviously have different compositions.[43] For the calculation of free energies from the ζ-potential, the assumption that only hydrophobic interactions are present is necessary. However, although this is the predominant one, it is probable that some electrostatic interactions are present, especially at low drug concentrations. Finally, it seems that drastic changes in the catalase structure at pH 10.0 are induced at high nafcillin concentrations.

Acknowledgment. The authors thank the Xunta de Galicia for financial support.

References and Notes

(1) Creighton, T. E.; Kim, R. S. *Curr. Opin. Struct. Biol.* **1991**, *1*, 3.
(2) Creighton, T. E. *Protein Folding*; W. H. Freeman: New York, 1992.
(3) Cassel, J.; Gallagher, J.; Reynolds, J.; Steinhardt, J. *Biochemistry* **1969**, *8*, 1706.
(4) Ibel, K.; May, R. P.; Kirschner, K.; Szadkowsky, H.; Mascher, E.; Lundahl, P. *Eur. J. Biochem.* **1990**, *190*, 311.
(5) Jones, M. N. In *Surface Activity of Proteins*; Magdassi, S., Ed.; Marcel Dekker: New York, 1996; Chapter 9, p 269.
(6) Sarmiento, F.; Prieto, G.; Jones, M. N. *J. Chem. Soc., Faraday Trans.* **1992**, *88*, 1003.
(7) Housaindokht, M. R.; Jones, M. N.; Newall, J. F.; Prieto, G.; Sarmiento, F. *J. Chem. Soc., Faraday Trans.* **1993**, *89*, 1963.
(8) Prieto, G.; del Rio, J. M.; Paz Andrade, M. I.; Sarmiento F.; Jones, M. N. *Int. J. Biol. Macromol.* **1993**, *15*, 343.
(9) Pombo, C.; Prieto, G.; del Rio, J. M.; Sarmiento F.; Jones, M. N. *Int. J. Biol. Macromol.* **1996**, *18*, 55.
(10) López-Fontán, J. L.; Rega, C.; Mosquera, V.; Sarmiento, F.; Jones M. N. *J. Chem. Termodyn.* **1999**, *31*, 1297.
(11) Jones, M. N.; Prieto, G.; del Rio, J. M.; Sarmiento, F. *J. Chem. Soc., Faraday Trans.* **1995**, *91*, 2805.
(12) Mosquera, V.; Ruso, J. M.; Prieto, G.; Sarmiento, F. *J. Phys. Chem.* **1996**, *100*, 16749.
(13) Sarmiento, F.; Ruso, J. M.; Prieto, G.; Mosquera, V. *Langmuir* **1998**, *14*, 5725.
(14) Taboada. P.; Mosquera, V.; Ruso, J. M.; Sarmiento, F.; Jones, M. N. *Langmuir* **2000**, *16*, 934.
(15) Taboada. P.; Mosquera, V.; Ruso, J. M.; Sarmiento, F.; Jones, M. N. *Langmuir* **2000**, *16*, 6795.
(16) Taboada, P.; Attwood, D.; Ruso, J. M.; García, M.; Sarmiento, F.; Mosquera, V. *Langmuir* **2000**, *16*, 3175.
(17) Deisseroth, A.; Dounce, A. L. *Physiol. Rev.* **1970**, *50*, 319.
(18) Schonbaum, G. R.; Chance, B. In *The Enzymes*; Boyer, P. D. Ed.; Academic: New York, 1976; Vol. 13 (part C9), pp 363−408.
(19) Sund, H.; Weber, K.; Molbert, E. *Eur. J. Biochem.* **1967**, *1*, 400.
(20) Pitt-Rivers, R.; Impiombato, F. S. A. *Biochem. J.* **1968**, *109*, 825.
(21) Nelson, C. A. *J. Biol. Chem.* **1971**, *246*, 3895.
(22) Takeda, A.; Hachimori, A.; Murai, M.; Sato, K.; Samejina, T. *J. Biochem.* **1975**, *78*, 911.
(23) Jones, M. N.; Manley, P. *Int. J. Biol. Macromol.* **1982**, *4*, 201.
(24) Jones, M. N.; Manley, P.; Midgley, P. J. W.; Wilkinson, A. E. *Biopolymers* **1982**, *201*, 1435.
(25) Nogueira, M.; Finn, A.; Jones, M. N. *Int. J. Biol. Macromol.* **1986**, *8*, 270.
(26) Vainshtein, B. K.; Melik-Adamyan, W. R.; Barynin, V. V.; Vagin, A. A.; Grebenko, A. I. *Nature* **1981**, *293*, 411.
(27) Hunter, R. J. *Zeta Potential in Colloid Science*; Academic Press: London, 1981; Chapter 3.
(28) Reid, T. J.; Murthy, M. R.; Sicignano, A.; Tanaka, N.; Musick, W. D.; Rossmann, M. G. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4767.
(29) Regelsberger, G.; Jakopitsch, C.; Rüker, F.; Krois, D.; Peschek, G. A.; Obinger, C. *J. Biol. Chem.* **2000**, *275*, 22854.
(30) Gebica, L. *Int. J. Biol. Macromol.* **1999**, *24*, 69.
(31) Kayes, J. B. *J. Colloid Interface Sci.* **1976**, *56*, 426.
(32) Stadilis, G.; Avranas, A.; Jannakoudakis, D. *J. Colloid Interface Sci.* **1990**, *135*, 313.
(33) Moosavi-Movahedi, A. A.; Housaindokht, M. R. *Physiol. Chem. Phys. Med. NMR* **1990**, *22*, 19.
(34) Jones, M. N.; Manley, P.; Holt, A. *Int. J. Biol. Macromol.* **1984**, *6*, 65.
(35) Jones, M. N.; Manley, P.; Midgley, P. J. W. *J. Colloid Interface Sci.* **1981**, *82*, 257.
(36) Hall, D. G. *J. Chem. Soc., Faraday Trans. 1* **1980**, *76*, 386.
(37) Sexsmith, F. H.; White, H. J. *J. Colloid Sci.* **1959**, *14*, 630.
(38) Murray, R. C.; Hartley, G. S. *Trans. Faraday Soc.* **1935**, *31*, 183.
(39) Mysels, K. J. *J. Colloid Sci.* **1955**, *10*, 507.
(40) Taqueda, A.; Hachimori, A.; Murai, M.; Sato, K.; Samejima, T. *J. Biochem.* **1975**, *78*, 911.
(41) Jones, M. N.; Chapman, D. *Micelles, Monolayers and Biomembranes*; Wiley-Liss: New York, 1995.
(42) Connors, K. A. *Binding Constants: The Measurements of Molecular Complex Stability*; Wiley: New York, 1987.
(43) Hunter, R. J. *Zeta Potential in Colloid Science*; Academic Press: London, 1981; Chapter 2, p 34.