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수용성 단백질의 계면상 등온곡선의 모델과 실험적 규명

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Model and Experimental Isotherms of Soluble Proteins at water surfaces

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ABSTRACT

A surface equation of state for globular proteins at air-water interface accounting for the molecular structure, segment-segment , segment-solvent, and electrostatic interactions was proposed and compared to C-14 isotope experiments. This lattice model comprised a simplifying assumption that all adsorbed segments are in the form of trains. The number of segment adsorbed per molecule in case of bovine serum albumin linearly depended on the surface concentration whereas the lysozyme segments adsorbed at the interface were independent of surface concentration. The segment-solvent(water) interaction for both of proteins were found to be unfavorable owing to the proteins unfolding. From comparison of model computation and experimental data, BSA unfolded more than lysozyme because of the larger surface area of contact.

Introduction

Protein adsorption at fluid-fluid interfaces is important for a proper understanding of its ability to stabilize foams and emulsions in a variety of applications. Adsorption of proteins at the gas-liquid interface leads to a lowering of the surface tension or an increase in the surface pressure. The relationship beteen the surface pressure and the surface concentration or density is usually referred to as the surface equation of state or surface isotherm. Although this relationship can be determined for some proteins, a satisfactory mathematical model did not exist in the literature for handy prediction of protein behavior in solutions because the proteins' properties are not fully understood. Proteins are complex macromolecules formed by the association of a large number of amino acids. In globular proteins, the amino acid residues are folded into spherical shapes. Upon adsorption at an interface these proteins unfold.

Singer derived an expression for the surface pressure of flexible polymers with all segments adsorbed and no interactions on the basis of statistical thermodynamics. This Singer's model was supplemented by Davies and Lopis, Frisch and Simha. They incorporated segment-segment interactions and formation of loops and trains into the model. Though the existing models can fairly describe the surface equation of state of random coiled, flexble proteins such as casein, they are not likely to fit to globular macromolecules such as BSA and lysozyme, model system in this work. The aim of this work is to develop a reasonable surface equation of state describing interfacial behavior of globular type proteins.

Model Development

Consider a 2-dimensional lattice consisting of Ns lattice sites. Each lattice is occupied by a segment of an adsorbed protein molecule. The protein has n segments and the number of adsorbed molecules are N2 whereas the number of solvent molecules (water) are N1. We know that the segemnts can be placed in many ways within n lattice sites. If z is the coodination number of the lattice, we have the segment placement ways as

$$w'_n(z) = \frac{nz\{(z-1)f\}^{n-2}}{2}$$

where f is a flexibility factor.

Hence the number of ways of putting N2 molecules (adsorbed protein) in the lattice will be

$$\Omega = \frac{\Pi (N_s/n - i)^{w_n(z)}}{N_2!} \quad \text{for i=0, } N_2\text{-1}$$

From the entropy of mixing of statistical thermodynamics we need

$$\Delta S = k\ln\Omega = kw\sum_{i=0}^{N_2-1} \ln(1-\frac{ni}{N_s}) + kN_2w\ln(\frac{N_s}{n}) - kN_2\ln N_2$$

Also the enthalpy of mixing can be expressed as

$$\Delta H = \chi\theta N_1 = \chi\frac{N_2 n}{N_s}N_1$$

where χ is the Flory-Huggins parameter and θ is the fraction of the surface occupied by the segments. Constitution of above two factors gives Gibbs Free Energy of Mixing, ΔF_{mix}.

$$\Delta F_{mix} = \Delta H - T\Delta S$$

Since the total free energy is the sum of mixing and electrical energy, we now have

$$\Delta F_t = \Delta F_{mix} + \Delta F_{el}$$

where considers Gouy-Chapman model of the electrical double layer.

The surface pressure Π is then related to the free energy with

$$\Pi = -(\frac{d\Delta F_t}{dA})_{N_2,T} = f(\Gamma)$$

All the equations can be solved numerically.

Materials and Methods

BSA and lysozyme were purchased from Sigma Chemical Co. and stored at 4℃. They were used without further purification. 4x10-4 mmoles of BSA powder was dissolved in 12.5 ml of 0.05 M phosphate buffer. Then, 1.25 ml of 0.1 M NaCNBH3 was added to the solution. 10 ml of 2.1x10-4 M H14CHO stock solution (102 uCi) was mixed well with the protein solution. The reaction proceeded for 2 h at room temperature. After the reaction, the mixture was immediately put in a dialysis membrane (spectraPOR: MW curoff, 6000-8000) and was dialyzed for 30 h at 4 ℃. The dialized solution was dehydrated using PEG 8000. The final solution was kept in a freezer at -70 ℃ for preservation. A langmuir trough (330x75x6.5 mm3 from KSV) was used for adsorption experiments. The trough was equipped with Wilhelmy plate for surface pressure and a gas proportional detector (Ludlum Model 120 with 2x2 mylar window) for radioactivity detection. The radioactivity was measured under P-10 gas environment (55 ml/min) through the detector chamber and at 1450 V.

Results and Discussion

The infinite difference method was used to solve the problems for both planar and spherical geometries. Solution of the system was obtained from Patanka's tri-diagonal matrix algorithm (4).

With the kinetic adsorption parameters obtained from the constant-area experiments for 0.3 and 0.6 mM octanol solutions, certain mixed kinetics model for oscillating area experiments were calculated and compared with the data in Fig. 2. In all the examples, the observed amplitudes are larger than the ones predicted by the diffusion-controlled model. The pulsating-area results are qualitatively consistent with the constant-area data, requiring the use of a mixed kinetic model. The model with the Langmuir-Hinshelwood equation sometimes predicts higher amplitudes than the data, and sometimes predicts lower data. The case with the L-H equation matches the data well for 0.3 mM at 20 cycles/min. Some parameters we used such as Γ_m and k's, which was derived from the equilibrium state may cause this discrepancies. The discrepancies may also be due in part to the models not considering surface rheology as well as to measurement errors and impurities.

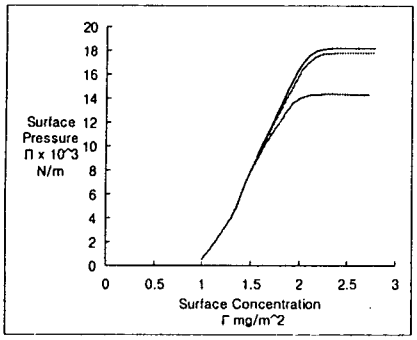

Fig. 1. Effect of variation in parameter a, which varies 0.68, 0.70, and 0.85. The other values are from Ref. 7.

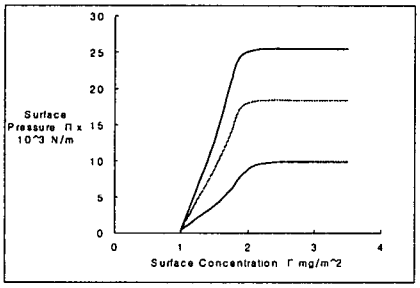

Fig. 2. Effect of pH in the surface equation of state. The other values are from Ref. 7.

Conclusion

A surface equation of state for globular proteins at air-water interface accounting for the molecular structure, segment-segment , segment-solvent, and electrostatic interactions was proposed and compared to C-14 isotope experiments. This lattice model comprised a simplifying assumption that all adsorbed segments are in the form of trains. The number of segment adsorbed per molecule in case of bovine serum albumin linearly depended on the surface concentration whereas the lysozyme segments adsorbed at the interface were independent of surface concentration. The segment-solvent(water) interaction for both of proteins were found to be unfavorable owing to the proteins unfolding. From comparison of model computation and experimental data, BSA unfolded more than lysozyme because of the larger surface area of contact.

References

1. Graham, D.E. and Phillips, M.C., J. colloid Interf. Sci., 70, 427 (1979)

2. Singer, S.J., J. Chem. Phys., 16, 872 (1948)

3. Davies, J.T. and Lopis, J.L., Proc. R. Soc. London A 227, 537 (1955)

4. Frisch, H.L., and Simha, R., J. Chem. Phys., 27, 702 (1957)

5. D. Cho, PhD Dissertation, Purdue University, 1996.

6. Jencroft, N. and Dearborn, D.G., Methods in Enzymol. 91, 570 (1983)

7. Means, G.E., Methods in Enzymol., 91, 469 (1983)

8. Verwey, E.J.W. and Overbeㄷㅏ, J. th G., Theory of the Stability of Lyophobic Colloids, Elsevier, NY. 1948.