Interaction between poly(ethylene glycol) and human serum albumin

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The thermodynamic nature of the interaction between polyethylene glycol and human serum albumin has been determined by affinity capillary electrophoresis.

Poly(ethylene glycol) (PEG) is of extreme interest in biomaterials chemistry because of its very unusual and unique lack of interaction with biological matter.[1] Surface bound PEG is known to reduce protein adsorption and has become a very high interest research area.[2] Major efforts are in progress to immobilize PEG and PEG-like molecules at surfaces by chemical grafting[3–7] and numerous coating and physical bonding schemes.[8–16] In addition, it has found extreme interest for use in one of the most challenging problems in biomaterials chemistry involving blood contact interactions with biomaterials. In this case, the clotting factor fibrinogen is not absorbed, the complement system is not activated, platelets are not bound and viral particles are not bound to PEG coated surfaces.[17] PEG's resistance to protein adsorption has been attributed to a steric stabilization effect, unique solution properties and its molecular conformation in aqueous solution.[1] However, the interaction that does occur between the protein and PEG is being used to stabilize proteins in solution and causes a decrease in thermal stability,[18] which have been attributed to steric exclusion[19] and binding to the nonpolar regions of the protein molecule,[20] respectively. It has been proposed that competition between steric expulsion forces and van der Waals attraction between the protein and PEG control the protein adsorption process. Although numerous theories and models have been proposed, there remains no consensus. Bergström *et al.*[2] found that very little fibrinogen adsorption occurs on surfaces treated with PEG in the molecular weight range from 1500 to 19 000 but that significant adsorption occurs if the molecular weights of the polymers fall below 1500 with 100% adsorption for 250 molecular weight PEG polymers. One explanation given is that the PEGs reject the protein because of an unfavourable entropy (negative entropy) change associated with compression of the configurationally random heavily hydrated PEG chains by the protein adsorption. There is, however, considerable ordering of water around the PEG chain since two or three waters are closely associated with each ethylene oxide unit.[21,22] Protein interaction would release some of this water of solvation and produce a positive entropy change. Their model discusses the need for the negative entropy of compression to be compensated by the latter and speculate that the enthalpy for water loss would be enthalpically unfavourable. In addition, the positive free energy change for this process would have to be overcome by other interactions for sorption to take place. It is clear that more information on the interaction between PEG and proteins is badly needed.

In recent experiments, we have used affinity capillary electrophoresis (ACE) to examine the interaction between PEG and human serum albumin (HSA) and have determined data that is extremely relevant to thermodynamic models that discuss entropic and enthaplic favorable and unfavorable scenarios. The ACE binding experiments were performed at 30 kV at 25, 40 and 60 °C using an open-tubular arrangement on a Hewlett-Packard Model G1600A system with HP^{3}D chemstation software. The PEG concentrations were 0, 0.500, 1.00, 2.50, 3.00, 4.00 and 7.00 mg ml^{-1}. The pH was maintained at 7.00 by a sodium phosphate buffer and the samples consisted of 30.0 μM human serum albumin. The neutral marker *N*,*N*-dimethylformamide served as a metric for the electroosmotic flow. The ACE method for measuring interaction energies and entropies of proteins and substrates involves measuring the change in electrophoretic mobility $\Delta\mu_{R,L}$ of the protein complex in the presence of various concentrations of ligand ([L]) such as PEG (23). Here $\Delta\mu_{R,L}$ is given by eqn. (1), where μ_R is the mobility

$$\Delta\mu_{R,L} = \mu_{R,L} - \mu_R \quad (1)$$

of the protein receptor and $\mu_{R,L}$ is the mobility of the protein–ligand complex. Form these values, Scatchard analyses can be performed using eqn. (2), where $\Delta\mu_{R,L,max}$ is the mobility of

$$\Delta\mu_{R,L}[L] = K_b\Delta\mu_{R,L,max} - K_b\Delta\mu_{R,L} \quad (2)$$

the protein receptor when it is saturated with ligand. Plotting $\Delta\mu_{R,L}[L]$ *vs.* $\Delta\mu_{R,L}$ gives a straight line with slope $-K_b$ and intercept of $K_b\Delta\mu_{R,L,max}$ and thus produces equilibrium constants. The typical Scatchard plot is shown in Fig. 1 for 25 °C. Measurements and similar treatments at 40 and 60 °C produce equilibrium constants that can be used in a second law treatment derived from the van't Hoff equation ($\ln K = -\Delta H/8.3145T + \Delta S/8.3145$) and plot a $\ln[K]$ *vs.* T^{-1} produces the enthalpy and entropy changes associated with the interactions. The second law plot is shown in Fig. 2. Here the enthalpy change was determined from the slope to be ΔH = 18.1 kJ mol^{-1} and from the intercept, the entropy change was determined to be ΔS = 166 J mol^{-1} K^{-1}. At room temperature, the free energy is calculated to be −31.4 kJ mol^{-1}.

These results show that the enthalpy of interaction is endothermic and that the interaction is entropically driven. This supports the conjecture that protein interaction would release some of the water of 'hydration' and produce a positive entropy change. The negative but small value of the free energy shows that the overall interaction is favorable but is not chemisorption or chemical binding but rather a process driven by other events. This information should help in formualting models of interaction between PEG and proteins and points to the fact that thermodynamic models must consider the entire system, protein, solvent and PEG.

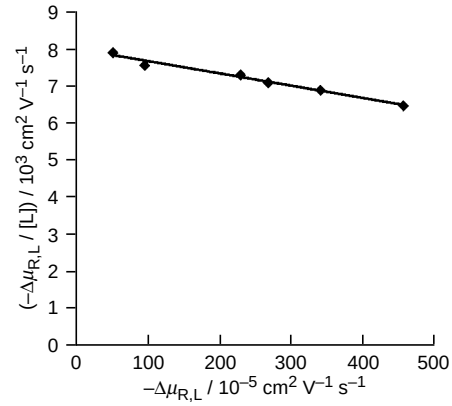

Fig. 1 ACE-derived Scatchard plot for the interaction of the HSA with PEG at 25 °C

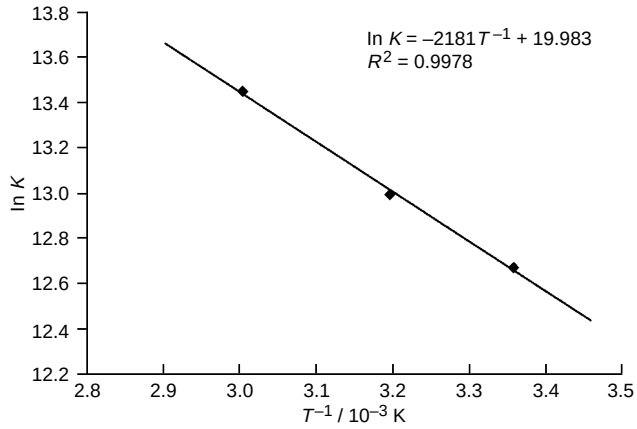

Fig. 2 Second law plot of ln K *vs.* T with the equation for the line and the variance

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Footnote and References

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