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Evidence that Water Can Reduce the Kinetic Stability of Protein−Hydrophobic Ligand Interactions

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Abstract: The first quantitative comparison of the thermal dissociation rate constants measured for protein−ligand complexes in their hydrated and dehydrated states is described. Rate constants, measured using surface plasmon resonance spectroscopy, are reported for the dissociation of the 1:1 complexes of bovine β-lactoglobulin (Lg) with the fatty acids (FA), palmitic acid (PA), and stearic acid (SA), in aqueous solution at pH 8 and at temperatures ranging from 5 to 45 °C. The rate constants are compared to values determined from time-resolved blackbody infrared radiative dissociation measurements for the gaseous deprotonated $(Lg+FA)^{n-}$ ions, where $n = 6$ and 7, at temperatures ranging from 25 to 66 °C. Notably, the hydrated (Lg+PA) complex is kinetically less stable than the corresponding gas phase $(Lg+PA)^{n-}$ ions at all temperatures investigated; the hydrated (Lg+SA) complex is kinetically less stable than the gaseous $(Lg+SA)^{n-}$ ions at temperatures <45 °C. The greater kinetic stability of the gaseous $(Lg+FA)^{n-}$ ions originates from significantly larger, by 11−12 kcal mol^{-1}, E_a values. It is proposed that the differences in the dissociation E_a values measured in solution and the gas phase reflect the differential hydration of the reactant and the dissociative transition state.

It is possible to gain insight into the effects of solvent on the rates of chemical reactions by comparing the kinetic parameters measured in the presence (solution) and absence (gas phase) of solvent. This general strategy has been used successfully to separate the influence of intrinsic properties and solvent on the rates of reactions involving small inorganic and organic molecules.[1] Given the ease with which noncovalent biological complexes can be transferred, intact and with at least partial retention of the native structure,[2] from aqueous solution to the gas phase with electrospray ionization (ES), there also exists an exciting opportunity to exploit this approach to characterize kinetic solvent effects in biological interactions. Here, we describe the first quantitative comparison of the thermal dissociation rate constants measured for protein−ligand complexes in their hydrated and dehydrated states. The kinetic parameters determined for ligand loss from two protein−fatty acid complexes suggest that water preferentially stabilizes the dissociative transition state with the result that the interactions are less stable in solution than in the gas phase.

The interaction between bovine β-lactoglobulin (Lg) and the fatty acids (FA), $CH_3(CH_2)_{14}COOH$ (palmitic acid ≡ PA) and $CH_3(CH_2)_{16}COOH$ (stearic acid ≡ SA), served as model protein−hydrophobic ligand complexes for this study. Lg, which exists as a monomer under alkaline conditions, possesses a large cavity composed of hydrophobic residues. The structure and thermodynamics of the interactions between Lg and a variety of hydrophobic ligands, including FAs, have been characterized. Competitive fluorescence measurements demonstrated that Lg exhibits a relatively high affinity for PA ($(5.0 \pm 0.2) \times 10^5$ M^{-1}) and other long chain FAs at pH 8.3 and 25 °C.[3] Analysis of the crystal structure reported for the (Lg + PA) complex (PDB 1B0O) reveals that the acyl chain of PA is fully buried within the hydrophobic cavity, while the carboxyl group is located near the top of the cavity.[4] Based on the crystal structure, the carboxyl group forms hydrogen (H)-bonds with Lys60 and Lys69 on β-strands C and D. However, the results of solution nuclear magnetic resonance measurements indicate that the position of the carboxyl group of bound PA fluctuates, suggesting that it does not participate in strong H-bonds with Lg.[5]

To our knowledge, dissociation kinetics for the (Lg+FA) complexes in aqueous solution have not been reported. In the present study, dissociation rate constants (k) were measured for the (Lg+PA) and (Lg+SA) complexes using surface plasmon resonance (SPR) spectroscopy at pH 8 and temperatures ranging from 5 to 45 °C. To perform the SPR measurements, Lg was immobilized to a carboxymethyl dextran surface through standard amine coupling chemistry. The Lg surfaces demonstrated nearly 100% activity based on PA saturation levels. Furthermore, the association constant (2.5×10^5 M^{-1}) measured by SPR spectroscopy for PA at pH 8 and 25 °C is in good agreement with the reported value.[3] These results confirm that immobilization does not alter appreciably the binding properties of Lg. The kinetic data for the dissociation of the (Lg+PA) complex, which can be described with single exponential functions, are shown in Figure S1a, Supporting Information. Similar results were obtained for SA, Figure S1b. Shown in Figure 1 are the Arrhenius plots determined for the dissociation of the (Lg+PA) and (Lg+SA) complexes. The Arrhenius parameters (E_a, A) as well as the activation parameters ($\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$) are listed in Table 1. Inspection of Figure 1 reveals that the (Lg+SA) complex, which exhibits a larger E_a (by 0.6 kcal mol^{-1}), is significantly more stable (kinetically) than (Lg+PA). The larger E_a can be understood in terms of the additional protein−lipid interactions arising from the extra $-CH_2-$ groups. The very small A-factors (<10^6 s^{-1}) indicate that dissociation occurs with a significant loss of entropy in the transition state (TS). The loss of entropy likely reflects a loss of protein configurational entropy, which is consistent with solution NMR results,[5] combined with the restriction of water molecules in the TS, *vide infra*.

Recently, it was shown that (Lg+FA) complexes composed of PA and SA are readily transferred from aqueous solution to the gas phase by ES and detected using mass spectrometry (MS).[6]

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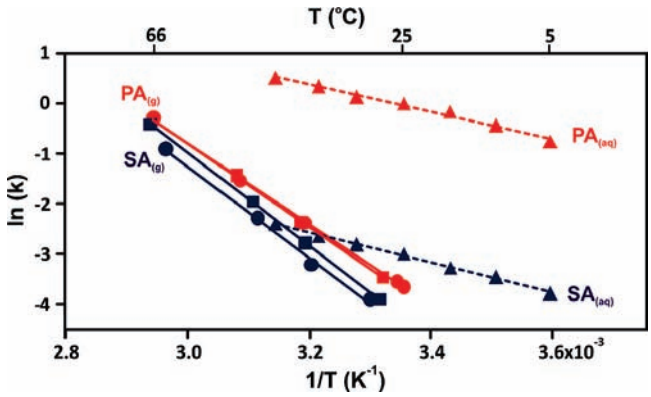

Figure 1. Arrhenius plots for loss of FA from the (Lg + FA) complexes, where FA = PA (red ▲) or SA (black ▲), measured in solution at pH 8, and from the gas phase deprotonated $(Lg+FA)^{n-}$ ions (*fast* components only), where FA = PA at $n = 6$ (red ■) and 7 (red ●) or SA at $n = 6$ (black ■) and 7 (black ●).

Table 1. Arrhenius Parameters (E_a, A) and Corresponding $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ Values for the Dissociation of (Lg+FA) Complexes in Solution (pH 8.0) and the *Fast* Components of the Gas Phase $(Lg+FA)^{n-}$ Ions at $n = 6$ and 7[a]

FA	E_a (kcal mol^{-1})	A (s^{-1})	$\Delta H^{\ddagger}$ [b] (kcal mol^{-1})	$\Delta S^{\ddagger}$ [b] (cal mol^{-1} K^{-1})
		Solution		
PA	5.4 ± 0.4	$10^{4.3\pm0.1}$	4.9 ± 0.4	−42
SA	6.0 ± 0.3	$10^{5.6\pm0.1}$	5.4 ± 0.3	−46
		Gas phase		
PA (−6)	16.6 ± 0.3	$10^{10.6\pm0.2}$	16.0 ± 0.3	−12
PA (−7)	16.2 ± 0.3	$10^{10.2\pm0.2}$	15.6 ± 0.3	−14
SA (−6)	18.5 ± 0.1	$10^{11.7\pm0.1}$	17.9 ± 0.1	−7
SA (−7)	18.0 ± 0.6	$10^{11.3\pm0.4}$	17.4 ± 0.6	−9

[a] The reported errors are 1 standard deviation. [b] Values at 298 K calculated from the corresponding Arrhenius parameters.

Furthermore, the affinity of the (Lg+PA) complex determined directly by ES-MS in negative ion mode[7] is in excellent agreement with the reported value,[3] suggesting that the (Lg+PA) complex remains intact in the gas phase. Thermal rate constants, determined using the blackbody infrared radiative dissociation technique, were reported for the loss of neutral FA from deprotonated $(Lg+PA)^{7-}$ and $(Lg+SA)^{7-}$ ions.[6] In the present study, the kinetic measurements were extended to $(Lg+PA)^{6-}$ and $(Lg+SA)^{6-}$ ions, Figure S2. As described elsewhere,[6] the gaseous $(Lg+FA)^{n-}$ ions adopt one of two kinetically distinct structures, referred to as the *fast* and *slow* components, which differ by the position of the flexible EF loop of Lg. In the *fast* component, the FA is stabilized predominantly by protein−lipid interactions, while, for the *slow* component, H-bonds between the ligand carboxyl group and Lg also contributes to the stability of the complex. Included in Figure 1 are the Arrhenius plots corresponding to the dissociation of the *fast* components of the $(Lg+FA)^{n-}$ ions; the Arrhenius and activation parameters are listed in Table 1. The energetic stability of the $(Lg+PA)^{n-}$ and $(Lg+SA)^{n-}$ ions, which are similar for the two charge states investigated, mirrors the trend observed in solution with the $(Lg+PA)^{n-}$ ions being less stable (by 2 kcal/mol). The lower E_a reflects the smaller acyl chain and the smaller number of

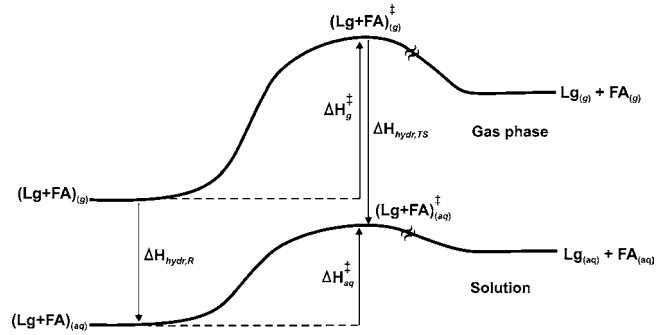

Figure 2. Energy diagram for the dissociation of the (Lg+FA) complex in aqueous solution and in the gas phase.

protein−lipid interactions. As described previously, the small A-factors suggest a loss of Lg configurational entropy in the TS.[6]

The most striking result of the present study is the greater stability of the desolvated (Lg+FA) complexes. It has been suggested that desolvation of hydrophobic protein interactions will result in a reduction in stability.[8] However, this is evidently not the case here. The greater kinetic stability of the gaseous $(Lg+FA)^{n-}$ ions (at temperatures < 45 °C) originates from the significantly larger, by 11−12 kcal mol^{-1}, E_a values. The differences in dissociation E_a values determined in the presence and absence of solvent may have several origins. It is possible that they reflect structural differences between the solvated and desolvated Lg, which affect the stabilizing intermolecular interactions. It is also possible that the gas phase E_a values are enhanced by ion−dipole or ion-induced dipole interactions (i.e., charge state effects). However, it is also possible that the differences in the E_a values simply reflect the influence of solvent on the dissociation reaction.

As illustrated in Figure 2, the difference in enthalpies of activation for identical protein−ligand dissociation reactions occurring in aqueous solution ($\Delta H^{\ddagger}_{aq}$) and the gas phase ($\Delta H^{\ddagger}_{g}$) reflects the difference in the hydration enthalpy of the TS ($\Delta H_{hydr,TS}$) and the reactant ($\Delta H_{hydr,R}$), eq 1a. In general, it is not possible to estimate the magnitude of the ΔH_{hydr} terms. However, the Lg system is unusual in that the binding cavity remains dry upon loss of the ligand. Furthermore, ligand dissociation does not result in significant changes in the Lg higher order structure.[9] Consequently, it is reasonable to expect that the $(\Delta H_{hydr,R} - \Delta H_{hydr,TS})$ term will be dominated by the hydration of the escaping ligand in the TS. In this case, the difference in $\Delta H^{\ddagger}$ values, which is equivalent to the difference in E_a values, can be approximated as the hydration enthalpy of the FA in the TS, eq 1b. Assuming a late TS, this would correspond to the hydration of the acyl chain of the FA. Values of $\Delta H_{hydr,FA}$ for PA (−11.7 kcal mol^{-1}) and SA (−13.0 kcal mol^{-1}) at 25 °C were calculated using hydration enthalpies tabulated for hydrocarbons.[10] Notably, the $\Delta H_{hydr,FA}$ values agree, within ∼1 kcal mol^{-1}, with the differences in the E_a values measured in solution and the gas phase. Although not definitive, the results of this analysis support the hypothesis that the differences in E_a values are due primarily to hydration effects.

$$\Delta H^{\ddagger}_{aq} = \Delta H^{\ddagger}_{g} + (\Delta H_{hydr,R} - \Delta H_{hydr,TS}) \quad (1a)$$

$$\Delta H^{\ddagger}_{aq} \approx \Delta H^{\ddagger}_{g} + (\Delta H_{hydr,FA}) \quad (1b)$$

In summary, the first quantitative comparison of the dissociation rate constants for protein−ligand complexes in their solvated

and desolvated states is described. Contrary to the popular belief that protein−hydrophobic ligand interactions are weakened in the gas phase, the hydrated (Lg+FA) complexes are less stable than the gaseous ions. It is proposed that the differences in dissociation E_a measured in solution and the gas phase reflect the differential hydration of the reactant and the dissociative TS. Clearly, however, data for additional protein−hydrophobic ligand complexes are needed to establish the generality of the current findings and to further elucidate the origin of the enhanced stability of the gaseous (Lg+FA) complexes investigated here.

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Supporting Information Available: Experimental methods and kinetic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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