Biological Complexes

Partitioning of Solvent Effects and Intrinsic Interactions in Biological Recognition**

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The association of molecules to form specific, noncovalent complexes is central to many biological processes. The strength and specificity of binding is governed both by the formation of selective solute–solute interactions and by solvent effects.[1–4] Although the structures and association thermochemistry of many biological complexes in solution have been determined, these data do not provide a complete description of the recognition process.[5] The challenge for researchers aiming to achieve a more complete understanding of the molecular recognition process is the separation of solvent effects from solute–solute interactions, something never-before accomplished for a biomolecular complex. Herein, we describe a novel methodology to perform this task. Our approach involves comparison of the energetic stabilities of solvated complexes with those of their desolvated (gaseous) counterparts.

The enthalpy of association in solution (ΔH_{assoc}) can be expressed as the sum of the enthalpic contributions made by intrinsic solute–solute interactions (ΔH_{intrin}) and solvent effects (ΔH_{solv}), as shown in Equation (1).

$$\Delta H_{assoc} = \Delta H_{intrin} + \Delta H_{solv} \quad (1)$$

The ΔH_{assoc} term can be determined directly by isothermal titration calorimetry (ITC)[6] or from a van't Hoff analysis of the temperature dependence of the association constant.[7] The strength of the solute–solute interactions is most reliably determined in the absence of solvent, that is, in vacuo. In principle, ΔH_{intrin} can be determined from the temperature dependence of the equilibrium constant for the association of the complex in the gas phase, that is, from the slope of a van't Hoff plot. This slope corresponds to $\Delta H_{assoc,g}$, the association enthalpy of the gaseous complex. However, such measurements are generally not possible for biologically relevant molecules because of their extremely low vapor pressures. Furthermore, measurements of this kind probably would not provide the desired thermodynamic information since the structure of the complex formed in the gas phase is unlikely to resemble the structure in solution.[8] A more useful quantity is the energetic stability of the biomolecular complex formed initially in solution and then dehydrated such that it retains the essential aspects of its solution structure. Transfer of biomolecular complexes from the aqueous phase to the gas phase by electrospray or nanoelectrospray ionization readily affords the desolvated species. The value of $\Delta H_{assoc,g}$ cannot be determined directly but can be estimated from the Arrhenius activation energy (E_a) for the dissociation of the desolvated complex at thermal equilibrium. The value E_a is related to the enthalpy of activation for the dissociation of the complex, $\Delta H^{\ddagger}_{diss,g}$ ($E_a = \Delta H^{\ddagger}_{diss,g} + RT$),[9] which can provide a good approximation of the magnitude of $\Delta H_{assoc,g}$ (opposite sign to that of $\Delta H^{\ddagger}_{diss,g}$) if the reaction is assumed to be barrierless (that is, no reverse activation energy).[10] If the reaction is not barrierless, determination of $\Delta H^{\ddagger}_{diss,g}$ yields an overestimation of $\Delta H_{assoc,g}$. Providing that the solution structure is preserved in the gas phase, the contribution of ΔH_{solv} to the formation of a complex is simply the sum of the experimentally determined ΔH_{assoc} and $\Delta H^{\ddagger}_{diss,g}$ values [Eq. (2)].

$$\Delta H_{assoc} = \Delta H_{intrin} + \Delta H_{solv} = \Delta H_{assoc,g} + \Delta H_{solv} \approx -\Delta H^{\ddagger}_{diss,g} + \Delta H_{solv} \quad (2)$$

NMR spectroscopy and X-ray crystallography can be used to gain an insight into the structures of biomolecular complexes in solution. However, no techniques are available that allow direct characterization of the structure of large gaseous ions, so identification of the individual intermolecular interactions in gaseous biological complexes has not been possible. Our research group recently developed a novel reactivity-based approach that employs blackbody infrared radiative dissociation (BIRD),[10,11] a thermal dissociation technique implemented with a Fourier-transform ion cyclotron resonance mass spectrometer. We used a combination of BIRD and functional group replacement (FGR) to identify and quantify individual interactions in gaseous biomolecular complexes.[12,13] The main features of this approach are described below.

The potential energy surface for the dissociation of a gaseous biomolecular complex stabilized by multiple noncovalent interactions typically has a "staircase" appearance (Figure 1). Each step corresponds to the cleavage of an intermolecular interaction.[12] Dissociation of the complex proceeds by sequential cleavage of the intermolecular interactions. The transition state (TS) corresponds to the structure

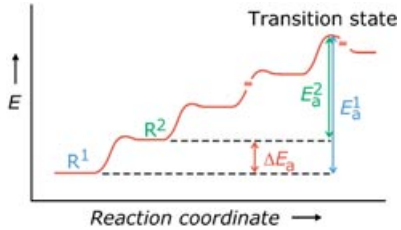

Figure 1. Energy diagram for the sequential cleavage of the intermolecular hydrogen bonds stabilizing two noncovalent complexes, R^1 and R^2, which differ by a single interaction. The strength of this interaction is equal to the difference in dissociation activation energy (E_a) between R^1 and R^2, that is, $\Delta E_a = E_a^1 - E_a^2$.

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formed during cleavage of the last remaining interaction. The thermodynamic formulation of transition-state theory states that the unimolecular reaction rate constant (k) reflects the enthalpy (or energy) difference between the TS and the reactant (R), in which all interactions are present [Eq. (3a,b); k_B is the Boltzmann constant, h is Planck's constant, and $\Delta S^{\neq}$ is the entropy of activation].

$$k = (k_B T/h)\exp(\Delta S^{\neq}/R)\exp(-\Delta H^{\neq}/RT) \quad (3a)$$

$$k = (k_B T/h)\exp(1+\Delta S^{\neq}/R)\exp(-E_a/RT) \quad (3b)$$

In the absence of extensive intramolecular solvation of groups originally involved in intermolecular interactions, E_a (determined from the temperature dependence of k) reflects the sum of *all* individual interactions in the reactant. The contribution of individual functional groups to the stability of the complex can be determined from the difference between the E_a values of structurally related complexes that differ by a single interaction. A change (decrease) in E_a coinciding with a structural modification (that is, $\Delta E_a = E_a$(unmodified complex)$-E_a$(modified complex)$\neq 0$) indicates that the modified group was involved in stabilizing the complex. If the structural modification eliminates a particular intermolecular interaction, ΔE_a provides a measure of the strength of the lost interaction.

A limited number of structural studies on gaseous noncovalent protein–ligand complexes have provided us with evidence that nonspecific interactions (interactions not present in solution that form during the desolvation process) can influence stability.[12,14] A modified methodology that focuses on the energies of individual interactions rather than on the overall energy of the entire collection of interactions is therefore more appropriate. Such a *single interaction* approach, while experimentally labor intensive, has the important advantage that it minimizes the influence of systematic error upon measurements (both solution and gas phase) because the quantities of interest are derived directly from the difference between two experimental values.

The contribution of a given solution-specific intermolecular interaction to the overall ΔH_{assoc} value can be determined by measuring the change in ΔH_{assoc} that results from the loss of the interaction, that is, $\Delta\Delta H_{assoc} = \Delta H_{assoc}$(unmodified complex)$-\Delta H_{assoc}$(modified complex). This approach is rigorously correct only when the individual interactions operate in an additive fashion but should provide a good approximation of the enthalpic contribution of specific interactions and is widely used in association thermochemistry to assess the contributions of particular functional groups or residues.[15] As outlined above, the strength of individual interactions in the gaseous complex is readily established from the ΔE_a values determined with the BIRD/FGR method. The enthalpy change resulting from solvent reorganization upon formation of a specific solute–solute interaction ($\Delta\Delta H_{solv}$) can be estimated as the difference between the change in binding enthalpy (energy) upon loss of the interaction in solution ($\Delta\Delta H_{assoc}$) and that in the gas phase ($-\Delta E_a$), as shown in Equation (4).

$$\Delta\Delta H_{assoc} = \Delta\Delta H_{intrin} + \Delta\Delta H_{solv} \approx -\Delta E_a + \Delta\Delta H_{solv} \quad (4)$$

We used this methodology to determine $\Delta\Delta H_{solv}$ for one of the specific hydrogen bonds in the complex formed by a single-chain variable domain fragment (scFv) from the carbohydrate-binding IgG antibody Se155-4 with its native trisaccharide ligand, Galα[Abe]Man (**1**). The complex of **1** with the Se155-4 antibody was the focus of a comprehensive study on carbohydrate recognition by proteins in aqueous solution. The crystal structure of the related scFv·**1** complex has been solved[16] (Figure 2) and the association thermochemistry of the complexation of this IgG with **1**, as well as with several monodeoxy congeners, has been determined by ITC.[17] The scFv·**1** complex is among the biomolecular

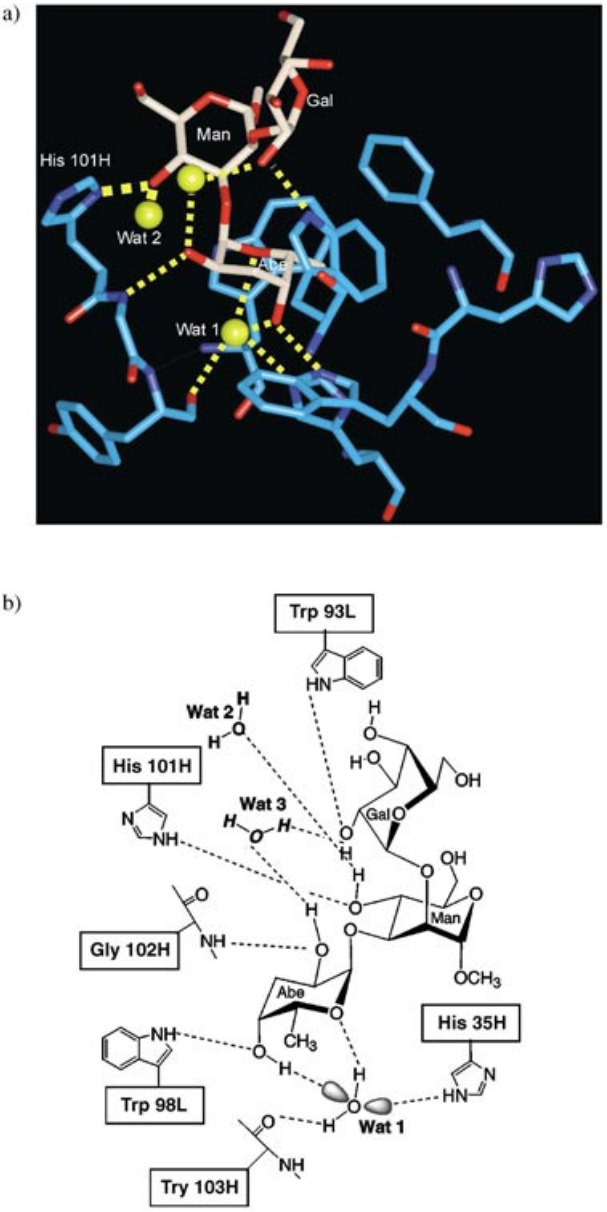

Figure 2. Crystal structure of the scFv·Galα[Abe]Man complex (scFv·**1**). a) Structure of the complex formed by the single-chain antibody fragment and the trisaccharide ligand Galα[Abe]Man. The H_{His101}–OH_{ManC4} hydrogen bond and the solvent-exposed nature of the mannose (Man) residue compared to the buried abequose (Abe) residue are shown. b) Detailed hydrogen-bonding map for scFv·**1**. Wat: water molecule.

complexes most extensively investigated in the gas phase.[8,12,13,18] A previous gas-phase study of the scFv·**1** complex and complexes of structural analogues (produced by nanoelectrospray ionization) showed that the specific hydrogen bond H_{His101}–OH_{ManC4} present in solution (Figure 2) is preserved in the gas phase.[13] This is the first and, at present, only example of the preservation of a specific intermolecular interaction in a gaseous biomolecular complex.

The ΔH_{assoc} values of the complexes formed by scFv with **1** and with the Man C4 monodeoxy congener **2**, in which the H_{His101}–OH_{ManC4} hydrogen bond is absent, were determined by ITC. From these values, we calculated that $\Delta\Delta H_{assoc} = 1.7 \pm 0.3$ kcal mol^{-1} for the H_{His101}–OH_{ManC4} interaction.[16] In principle, the energy of the H_{His101}–OH_{ManC4} hydrogen bond determined for a single charge state of the scFv·**1** complex could be used in conjunction with the $\Delta\Delta H_{assoc}$ value to arrive at a value for $\Delta\Delta H_{solv}$. However, it is possible that electrostatic effects arising from the multiple charges on the gaseous complex influence the interaction. To establish that charge effects are not significant, we applied the BIRD/FGR approach to evaluate the H_{His101}–OH_{ManC4} interaction energy over a range of charge states (+6 to +10 and −8). The strength of the interaction was determined in three ways for each charge state: 1) by using functional group modification, whereby the interaction energy is determined from the difference between the E_a value of the scFv complex with **1** and that of the complex with the monodeoxy congener **2** (that is, ΔE_a), 2) active site mutation, whereby the interaction energy is determined from the ΔE_a value for the complexes formed by **1** with scFv and with a His101Ala mutant in which the His101 residue is replaced with alanine, and 3) pair-wise modification, whereby the interaction energy is determined from the ΔE_a value for the complex formed by scFv with **1** and that formed by the His101Ala mutant with **2**. Table 1 shows that we determined the same hydrogen-bond strength for each of the six charge states with all three modification strategies, within the precision of the measurements. The fact that the hydrogen-bond strength remains the same for all of the protonated and deprotonated ions implies that electrostatic effects do not significantly influence the interaction, at least at these low charge states. The average interaction energy determined for the six charge states by using the three modification strategies is 3.6 ± 1.2 kcal mol^{-1}. This value is lower than that calculated for a single hydrogen bond in an imidazole–H_2O complex (6 kcal mol^{-1}), which is the best available model for the H_{His101}–OH_{ManC4} interaction.[19] The lower energy calculated for the H_{His101}–OH_{ManC4} interaction may reflect the effect of steric or conformational structural constraints, which prevent optimal bonding.

Table 1: The differences between the Arrhenius activation energies [kcal mol^{-1}] measured upon loss of a neutral ligand from gaseous protonated and deprotonated scFv·**1** (I), His101Ala·**1** (II), scFv·**2** (III), and His101Ala·**2** (IV) in six charge states.

Charge state	ΔE_a (I)−(II)[a]	ΔE_a (I)−(III)[a]	ΔE_a (I)−(IV)[a]
+6	2.7±1.8	4.9±1.4	3.9±1.9
+7	4.1±1.1	4.0±2.1	3.0±1.6
+8	2.8±1.8	1.8±1.7	2.9±1.6
+9	4.7±1.7	1.9±1.6	2.7±1.0
+10	4.3±1.0	5.2±1.6	4.6±1.2
−8	3.1±1.3	3.8±1.0	3.9±1.4
Average ΔE_a[b]	3.6±1.0	3.6±1.5	3.5±1.0
$\Delta\Delta H_{solv}$[c,d]	5.3±1.3	5.3±1.7	5.2±1.3

[a] Errors were calculated by propagation of one standard deviation of the corresponding E_a values. [b] Mean values ± standard deviations for the six measurements. [c] $\Delta\Delta H_{solv}$ values [kcal mol^{-1}] calculated for the H_{His101}–OH_{ManC4} interaction from the ΔE_a and ΔH_{assoc} values determined for I and III by ITC: $\Delta\Delta H_{solv} = \Delta\Delta H_{assoc} + \Delta E_a$, where $\Delta\Delta H_{assoc} = 1.7 \pm 0.3$ kcal mol^{-1}.[14] [d] Errors were calculated by propagating one standard deviation of the $\Delta\Delta H_{assoc}$ and ΔE_a values.

The energetic data obtained in solution ($\Delta\Delta H_{assoc} = 1.7 \pm 0.3$ kcal mol^{-1}) and in the gas phase ($\Delta E_a = 3.6 \pm 1.2$ kcal mol^{-1}) were combined by using Equation (4) to give $\Delta\Delta H_{solv} = 5.3 \pm 1.4$ kcal mol^{-1} for the formation of the H_{His101}–OH_{ManC4} hydrogen bond. We believe that the magnitude of $\Delta\Delta H_{solv}$ corresponds to the net enthalpy change arising from the displacement of water molecules from the Man C4 hydroxy group and the His101 imidazole NH group, and the return of these water molecules to the bulk solution. Since our measurement is the first of its kind, it is not possible to compare our result with other measurements. However, the enthalpies of hydration of small model compounds have been determined calorimetrically and used to evaluate the enthalpies of hydration of various functional groups.[20] These values provide an estimate of the hydration enthalpy of polar and nonpolar groups associated with protein unfolding[20] and it is of interest to compare them with the $\Delta\Delta H_{solv}$ value determined in the study reported herein. The average enthalpies of hydration for OH and NH groups in small, linear molecules are −9.5 and −9.2 kcal mol^{-1}, respectively. The enthalpic penalty for completely dehydrating both an NH and an OH group is approximately 19 kcal mol^{-1}, a value roughly four times larger than our calculated $\Delta\Delta H_{solv}$ value. We believe the difference in these values has two origins: 1) The scFv·**1** crystal structure shows that the C4 OH group of Man is not fully dehydrated but remains associated with a single water molecule; 2) the chemical environments of the NH and OH groups in the protein and trisaccharide ligand are different from those in the small model systems. In the protein and trisaccharide, neighboring group effects may alter and reduce the strength of interactions between the NH and OH groups and solvent molecules. Consequently, 19 kcal mol^{-1} represents an upper limit for the dehydration enthalpy. This analysis highlights the dangers associated with the use of thermochemical parameters established for small, model systems to predict parameters for large, complex biological molecules.

The methodology described herein may be applied to other classes of biomolecular complexes and will allow the determination of solvent effects according to type of interaction and chemical environment. This information is imperative for the development of a molecular model of biological recognition. Such models could, in turn, lead to a paradigm shift in rational drug design and may aid the development of predictive models for protein folding.

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