Determination of Protein−Ligand Association Thermochemistry Using Variable-Temperature Nanoelectrospray Mass Spectrometry

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Electrospray ionization mass spectrometry (ES/MS) is a powerful tool for studying noncovalent biological complexes such as multiprotein assemblies and protein−ligand complexes. In addition to providing a rapid and sensitive method for detecting biomolecular complexes and directly establishing their binding stoichiometry, ES/MS-based methods hold tremendous promise for quantifying the noncovalent interactions. There are a number of reported examples where it has been shown that the relative abundance of unbound and bound species (e.g., protein/protein−ligand complex) measured by ES/MS reflects, quantitatively, their relative concentrations in solution at equilibrium.[1−3] From a "snapshot" of the solution composition and known initial concentrations, it is possible to evaluate the association constant (K_{assoc}). Although most ES/MS affinity measurements reported to date have been performed at ambient temperatures, several studies have shown ES/MS to be suitable for studying the influence of temperature on association.[4] Here, we describe a novel temperature-controlled nanoelectrospray (nanoES) device which we have applied to a series of protein−carbohydrate complexes. We show, for the first time, that "variable-temperature" nanoES-MS can provide accurate protein−ligand binding affinities over a range of solution temperatures. We further demonstrate that the thermodynamic parameters for association, ΔH_{assoc} and ΔS_{assoc}, can be evaluated from the MS-derived K_{assoc} values.

Values of K_{assoc} for carbohydrate complexes of two carbohydrate-binding proteins were measured over a range of temperatures. Measurements were performed on the complexes of a single chain variable fragment (scFv) of the monoclonal antibody Se155-4[5] with its native trisaccharide ligand, Galα[Abe]Man (**1**), and the structural analogues: AbeMan (**2**), Talα[Abe]Man (**3**), Glc2[Abe]Man (**4**), and Glc2Gal[Abe]Man (**5**). A second system investigated was the complex of the homopentameric binding subunit of Shiga-like toxin 1 (SLT-1B5 ≡ B5) with the P^k trisaccharide (**6**), the carbohydrate portion of the natural cell-surface receptor, the glycolipid Gb3.[6] From the temperature dependence of K_{assoc}, values of ΔH_{assoc} and ΔS_{assoc} were determined and compared to values obtained by isothermal titration calorimetry (ITC).

NanoES mass spectra were acquired, in positive ion mode, from aqueous solutions of protein, carbohydrate ligand, and CH_3COONH_4 at temperatures in the range of 5−40 °C. The temperature of the nanoES solution was regulated using a specially constructed nanoES device, which was attached to the front of the existing ion source of the 4.7 T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer used in this work.[7] The device consists of a Plexiglas chamber, which surrounds the sampling capillary of the mass spectrometer. The chamber is lined with a Cu sleeve, which is in thermal contact with a Cu coil that can be heated or cooled by circulating air. A portion of the temperature-regulated air is introduced into the chamber and satisfies the gas intake requirement (~600 mL/min) of the ion source. Using this dual approach, the temperature inside the chamber can be controlled to within ±1.5
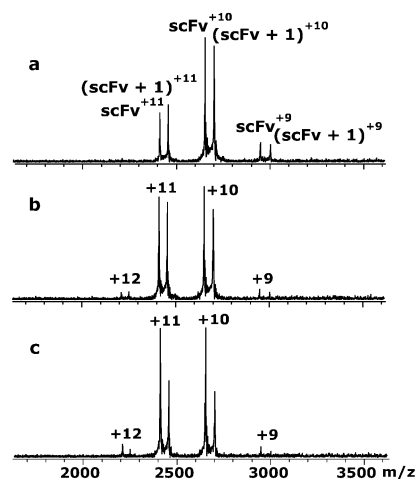

Figure 1. NanoES mass spectra obtained in positive ion mode for an aqueous solution of scFv (4 μM) and **1** (7 μM) with 2 mM CH_3COONH_4 at (a) 15 °C, (b) 25 °C, and (c) 35 °C.

°C. A diagram of the chamber (Figure S1) and a complete description of the instrumental parameters are included as Supporting Information.

Shown in Figure 1 are nanoES mass spectra acquired for a solution of scFv and **1** at 15, 25, and 35 °C. The ion abundance ratio of complex (scFv·**1**) to protein (scFv) is clearly seen to decrease with increasing solution temperature, which is the expected behavior because the association reaction is exothermic.[8] At a given solution temperature, K_{assoc} was calculated from the ion abundance ratio measured by nanoES-MS using a procedure described previously.[3]

The MS-derived values of K_{assoc} obtained for the scFv·**1** complex at temperatures ranging from 10 to 35 °C are shown in Figure 2 in the form of a van't Hoff plot. Each value of K_{assoc} represents the average of four measurements. Also shown in Figure 2 are the values of K_{assoc} determined by ITC for the Se155-4 IgG·**1** complex.[8] Because the mass of the IgG·**1** complex exceeds the capabilities of the mass spectrometer used in this work, MS-derived values of K_{assoc} could not be determined. However, given the uniform mode of binding for the IgG and scFv proteins, values of K_{assoc} for the IgG complex should closely resemble those of the corresponding scFv complex. The MS-derived K_{assoc} values are found to be in reasonable agreement with the ITC values over the temperature range investigated. This result indicates that any change in the temperature of the nanoES droplets, as compared to that of the bulk solution, resulting from the evaporation process or sampling into the MS does not influence the original equilibrium distribution of scFv and scFv·**1**. This observation is consistent with our analysis of the kinetics of gas phase ion formation, as compared to the association/dissociation kinetics of the complex, which suggested that the lifetime of the droplet is sufficiently short (<40 μs) that
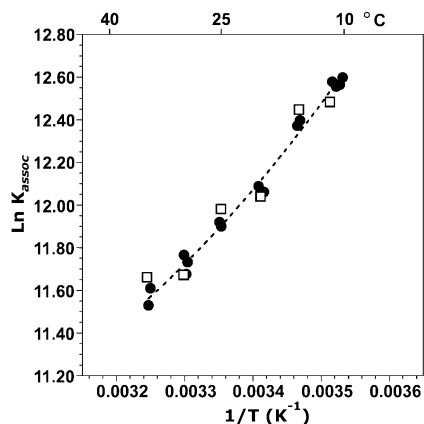
Figure 2. Temperature dependence of K_{assoc} for the reaction, scFv + **1** ↔ scFv·**1**, determined by nanoES-MS (●), and the reaction, IgG + **1** ↔ IgG·**1**, determined by ITC (□), ref 8. The dashed line corresponds to the nonlinear fit of the van't Hoff equation (eq 1) to the MS-derived values of K_{assoc}.

the original distribution of protein and complex is not altered by the nanoES process.[3]

The magnitude of ΔH_{assoc} and ΔS_{assoc} for the association of scFv and **1** was evaluated by subjecting the binding affinity data to a nonlinear least-squares analysis using the integrated form of the van't Hoff equation, which includes a temperature-independent heat capacity change (ΔC_p) (eq 1). T_o in eq 1 is an arbitrarily chosen reference temperature (taken to be 298 K in the present work), and K_o and ΔH_o are the association constant and enthalpy change, respectively, at that temperature. The magnitude of ΔS_{assoc} at T_o can then be calculated from the ΔH_o and ΔG_o (determined from K_o) values.

$$\ln \frac{K}{K_o} = \frac{\Delta H_o - T_o \Delta C_p}{R}\left(\frac{1}{T_o} - \frac{1}{T}\right) + \frac{\Delta C_p}{R}\ln \frac{T}{T_o} \qquad (1)$$

Fitting eq 1 to the MS data yielded a ΔH_{assoc} of 7.0 ± 0.3 kcal mol^{-1} at 25 °C. This agrees well with the value of 6.8 ± 0.4 kcal mol^{-1} established by ITC for the IgG·**1** complex.[8] It is worth noting that, despite a nonnegligible ΔC_p for this reaction, determined to be −114 cal mol^{-1} K^{-1} by ITC,[8] neglect of ΔC_p in the fitting procedure results in a ΔH_{assoc} of 7.4 ± 0.3 kcal mol^{-1}, which is indistinguishable from the value determined from the nonlinear fitting, within the experimental error.

To demonstrate that the nanoES/MS-based method is generally suitable for the determination of thermochemical parameters for protein−carbohydrate binding, values of K_{assoc} for five additional complexes were measured at temperatures of 5−40 °C. Van't Hoff plots obtained by MS for the association of the scFv and the ligands **2**−**5**, which are structural analogues of **1**, are shown in Figure S3. Also shown is the van't Hoff plot for the formation of the B_5·**6** complex. The thermochemical data obtained by nanoES-MS and ITC at 25 °C are listed in Table 1. The B_5 protein complex possesses five equivalent P^k binding sites, one per subunit. As a result, the apparent K_{assoc} value includes a statistical factor, which reflects the number of binding sites of the receptor.[9] The values of K_{assoc} (Figure S3) were adjusted to represent the P^k interaction at a single binding site. For all of the complexes investigated, the MS-derived ΔH_{assoc} values agree with the ITC results within 1 kcal mol^{-1}. Similarly, the MS-derived ΔS_{assoc} values are in reasonable agreement with the ITC results, within 4 cal mol^{-1} K^{-1}.

Table 1. Thermochemical Parameters for the Association Reaction: P + L ↔ (P·L), Where P = Protein (scFv, B_5) and L = Oligosaccharide Ligand (**1**−**6**), Determined at 25 °C by NanoES-FT-ICR/MS and ITC[a]

P	L	$-\Delta H_{assoc}$ (kcal mol^{-1}) MS	$-\Delta H_{assoc}$ (kcal mol^{-1}) ITC	$-\Delta S_{assoc}$ (cal mol^{-1} K^{-1}) MS	$-\Delta S_{assoc}$ (cal mol^{-1} K^{-1}) ITC
scFv	1	7.0 ± 0.3	6.8 ± 0.4[b]	−0.2 ± 0.2	−1.2 ± 1.6[b]
scFv	2	9.7 ± 0.4	9.3 ± 0.2[b]	8.7 ± 0.4	7.6 ± 0.6[b]
scFv	3	9.9 ± 0.8	9.1 ± 0.1[b]	10.0 ± 1.0	7.5 ± 0.2[b]
scFv	4	11.5 ± 0.5	11.2 ± 0.1[b]	12.3 ± 0.6	11.2 ± 0.3[b]
scFv	5	8.7 ± 0.8	8.0 ± 0.1[b]	3.7 ± 0.4	1.2 ± 0.1[b]
B_5	6	10.9 ± 1.1	12 ± 1[c]	23.0 ± 2.2	27 ± 1[c]

[a] The reported errors are one standard deviation. [b] Values taken from ref 8 and correspond to ligand binding to Se155-4 IgG. [c] Values taken from ref 6.

In summary, we have used a novel temperature-controlled nanoES device in a variable-temperature nanoES/MS study of protein−carbohydrate binding. Values of K_{assoc} were measured for solutions in the temperature range of 5−40 °C. From the temperature dependence of K_{assoc}, values of ΔH_{assoc} and ΔS_{assoc} (at 25 °C) were determined. The thermochemical data are found to be in good agreement with values determined previously by ITC. The results of this study demonstrate that variable-temperature nanoES-MS can be used to quantify the thermochemistry for protein−ligand binding. This technique is expected to find wide application in the areas of drug design and high throughput target screening.

Supporting Information Available: Description of the temperature-controlled nanoES device, experimental procedures, mass spectra, and van't Hoff plots (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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