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# Retention of Bioactive Ligand Conformation in a Gaseous Protein−Trisaccharide Complex

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Electrospray ionization mass spectrometry (ES-MS) is a powerful tool for studying solution-specific biomolecular complexes such as protein assemblies and protein−ligand complexes. Beyond the detection of complexes, ES-MS, in conjunction with gas-phase dissociation techniques, also holds considerable promise as a method for identifying binding sites and intermolecular interactions. Evidence for the preservation of specific intermolecular interactions in several gaseous protein−ligand complexes has been reported.[1a,b,2a] The formation of nonspecific interactions, however, has also been demonstrated.[1c,2b] A related and, arguably, more challenging problem is the elucidation of the conformation of binding partners in the gaseous complexes. Here, we describe results from thermal dissociation experiments performed on gaseous, protonated protein−trisaccharide complexes containing structurally preorganized trisaccharides, which indicate that aspects of the solution conformation of the ligand in the bound state can be preserved in the gas phase.

The complexes of a single-chain variable fragment (scFv) of the monoclonal antibody Se155-4[3] with its native trisaccharide ligand (L), Galα[Abe]Man (**1**), and the conformationally constrained trisaccharide ligands **2**, **3**, and **4** (Figure 1) served as model systems for this study. In solution, **1** is flexible and does not exist in any single, preferred conformation.[4] However, upon binding to the antibody, **1** undergoes a protein-induced conformational shift about the Gal-Man linkage.[4] According to the scFv·**1** crystal structure, the torsional angles Φ ($O_5-C_1-O_1-C_2$) and Ψ ($C_1-O_1-C_2-C_3$), for the Gal-Man glycosidic linkage, are 77 and 144°, respectively.[3] In **2**−**4**, intramolecular alkyl tethers ($C_6$ to $C_8$) attached to the C-6 oxygen of Gal and Man (residue **c** and **b**, respectively) restricts the motion about the Gal-Man glycosidic linkage so that they resemble the bioactive conformation. Molecular dynamics (MD) simulations indicate that unbound **2** samples a limited range of Gal-Man glycosidic linkage torsional angles (Φ 50−100°, Ψ 130−160°) in solution compared to **1** (Φ 40−110°, Ψ 70−160°).[5] Similar results were obtained from MD simulations performed on **1**−**4** in the gas phase at 400 K (see Figure S1, Supporting Information).

Using the blackbody infrared radiative dissociation (BIRD) technique[6] implemented with a Fourier transform ion cyclotron resonance mass spectrometer, the Arrhenius activation energies and preexponential factors, $E_a$ and $A$, for the dissociation of $(scFv + L)^{n+}$ complexes (eq 1), where $n = 10-12$, of **1** and the conformationally constrained trisaccharide ligands **2**, **3**, and **4** have been determined (Table 1).

$$(scFv + L)^{n+} \rightarrow scFv^{n+} + L \qquad (1)$$

For the +10 charge-state complexes, the dissociation $E_a$'s measured for **1**−**4** are indistinguishable, within experimental error (see Table 1). The $E_a$'s for the +11 and +12 charge-state complexes with the tethered ligands are lower than with **1**: $\Delta E_a = -3$ to $-4$ (**2**) and $\Delta E_a = -5$ to $-7$ kcal/mol (**3**,**4**). For all charge states, the complexes of the tethered ligands are kinetically more stable than the complex with **1**, and the stability increased with increasing tether length: **2** < **3** ≤ **4** (Figure 2).

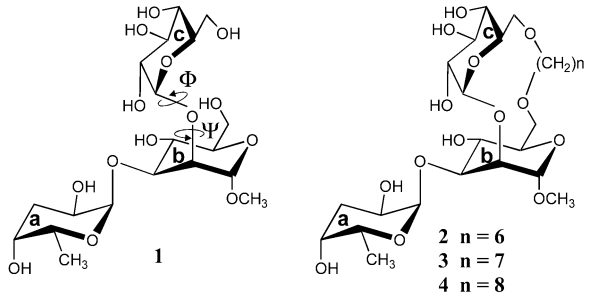


***Figure 1.*** Structures of the native trisaccharide ligand (**1**) and the tethered ligands (**2**−**4**).

***Table 1.*** Arrhenius Parameters Measured for the Reaction $(scFv + L)^{n+} \rightarrow ScFv^{n+} + L$, Where L = Trisaccharide (**1**−**4**)

| L | charge state | $E_a$ [a] (kcal/mol) | $\Delta E_a$ [b] (kcal/mol) | $A$ [a] ($s^{-1}$) | $\Delta S^{\ddagger}$ [d] (kcal/mol·K) |
|---|---|---|---|---|---|
| **1** | +**10** | 54.3 ± 1.0[c] | − | $10^{27.7\pm0.6}$ [c] | 65 |
| **2** | | 54.3 ± 1.0 | 0.0 | $10^{27.5\pm0.5}$ | 64 |
| **3** | | 54.1 ± 1.0 | −0.2 | $10^{27.2\pm0.5}$ | 63 |
| **4** | | 54.0 ± 1.3 | −0.3 | $10^{27.1\pm0.7}$ | 63 |
| **1** | +**11** | 52.1 ± 1.4 | − | $10^{26.9\pm0.8}$ | 61 |
| **2** | | 48.8 ± 1.2 | −3.3 | $10^{25.0\pm0.7}$ | 53 |
| **3** | | 45.7 ± 0.6 | −6.4 | $10^{23.1\pm0.3}$ | 44 |
| **4** | | 46.7 ± 0.5 | −5.4 | $10^{23.7\pm0.5}$ | 47 |
| **1** | +**12** | 47.0 ± 1.5 | − | $10^{24.3\pm0.8}$ | 49 |
| **2** | | 42.8 ± 0.8 | −4.2 | $10^{21.9\pm0.4}$ | 38 |
| **3** | | 40.2 ± 1.2 | −6.8 | $10^{20.4\pm0.6}$ | 32 |
| **4** | | 39.7 ± 1.0 | −7.3 | $10^{20.1\pm0.5}$ | 31 |

[a] The reported errors are one standard deviation. [b] $\Delta E_a = E_a$ (**2**, **3**, or **4**) $- E_a$ (**1**). [c] The Arrhenius parameters reported for the dissociation of $(scFv + 1)^{10+}$ were taken from ref 2a. [d] Values calculated for 415 K from the measured *A*-factors.

In two earlier studies, in which BIRD was applied to a series of structurally related $(scFv + L)^{n+}$ complexes, it was shown that the dissociation $E_a$'s are sensitive to the nature of the intermolecular interactions.[2] From the changes in $E_a$, which accompanied structural modification of the protein or ligand, it was possible to map the intermolecular interactions in the gaseous complex. A similar comparative approach is employed here to identify conformational changes in **1** accompanying the transfer of the scFv·**1** complex from solution to the gas phase. Specifically, the $E_a$'s measured for the complexes of the macrocyclic analogues **2**−**4** should be comparable to the $E_a$ for the complex with the bioactive form of **1**. The similarity in the $E_a$'s measured for all of the +10 charge-state complexes, therefore, strongly suggests that the complexes of all four ligands

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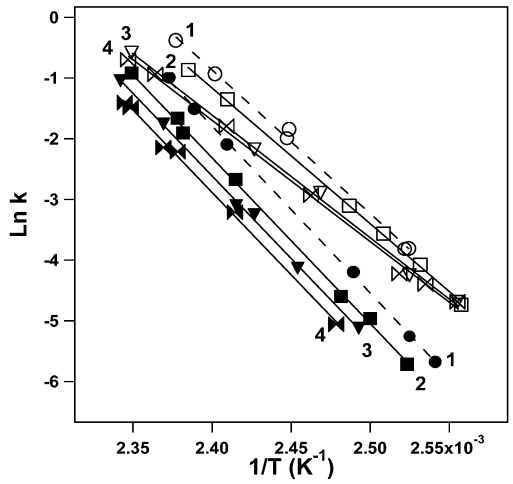


***Figure 2.*** Arrhenius plots for the dissociation of (scFv + L)$^{n+}$ complexes, where $n = 10$ (filled symbols), 12 (open symbols), with the native ligand (**1**) and the tethered ligands (**2**−**4**).

are stabilized by identical intermolecular interactions. This, in turn, is consistent with the conformation of **1** in (scFv + **1**)$^{10+}$ resembling the bioactive ligand conformation in scFv·**1**$_{(aq)}$. A network of intermolecular H-bonds that restrict conformational changes during desolvation can explain the retention of the solution ligand conformation in the gaseous complex. The H-bonds may originate in solution (i.e., specific) or they may form during the desolvation process (i.e., nonspecific). We have previously shown that the Gal C-3, C-6 and Man C-6 OH groups participate in nonspecific intermolecular H-bonds, while a H-bond at the Man C-4 OH group is specific.[2] Together, these interactions contribute ~33 kcal/mol of the energetic stability of the +10 complex[2b] and could effectively freeze in the bioactive conformer.

The kinetic data for the +10 charge-state complexes also provide new insight into the contribution of specific vibrational modes to the *A*-factor and entropy of activation ($\Delta S^{\ddagger}$) for the dissociation of the (scFv + L)$^{n+}$ complexes. We have previously proposed that the large *A*-factors, $>10^{20}$ s$^{-1}$, reflect the softening of numerous vibrational modes, associated with both the protein and the ligand, during the dissociation process.[2b] The smaller rate constants measured for the complexes of the tethered ligands, compared with that of **1**, are consistent with a reduction in the $\Delta S^{\ddagger}$ due to the restricted motion about the Gal-Man linkage. Assuming identical $E_a$'s (and $\Delta H^{\ddagger}$'s) for dissociation of the +10 charge-state complexes, the decrease in $\Delta S^{\ddagger}$ resulting from the presence of the tether, that is, $\Delta\Delta S^{\ddagger} = \Delta S^{\ddagger}(\mathbf{2}-\mathbf{4}) - \Delta S^{\ddagger}(\mathbf{1})$, was estimated from $\Delta\Delta G^{\ddagger}$, the difference in the free energy of activation, determined from the rate constants ($k(T) = (k_B T/h)\exp(-\Delta G^{\ddagger}/RT)$) measured at 415 K. Using this approach, $\Delta\Delta S^{\ddagger}$'s of −1 (**2**), −1.5 (**3**), and −2 cal/mol·K (**4**) are obtained. These results suggest that the vibrational entropy associated with the motion about the glycosidic linkages in di- and oligosaccharides in the gas phase is ≥2 cal/mol·K. Interestingly, the entropic penalty for the restriction of intersaccharide rotamers in solution at room temperature has been estimated to be in the range of 2−7 cal/mol·K.[5,7]

The decrease in $\Delta S^{\ddagger}$ with increasing tether length for the +10 complexes is intriguing and would not have been predicted from the MD simulations, which indicate that the longer tethers allow for greater conformational flexibility. The kinetic data suggest that the longer tethers participate in intramolecular interactions that constrain the motion about the Gal-Man glycosidic linkage but do not influence the intermolecular interactions and $E_a$.

The lower $E_a$'s measured for the +11 and +12 charge-state complexes with the tethers may be due to repulsion (steric effects) between the tether and the protein. However, this explanation seems unlikely since there was no such effect observed for the +10 complexes. Instead, charge-induced structural changes in the protein binding-site are believed to be responsible for the lower $E_a$'s. In a related study, the influence of charge on the stability of the (scFv·**1**)$^{n+}$ complex was studied using BIRD (unpublished results). The results suggested identical intermolecular interactions for the +7 to +10 charge states. However, at higher charge states, +11 to +13, electrostatic effects alter the interactions. Specifically, the H-bonds at Gal C-3 and Man C-4 and C-6 OH groups are weakened or lost, with the concomitant strengthening or formation of interactions elsewhere on the ligand. **1**, with its greater conformational flexibility, can adapt to these charge-induced structural changes and maximize the interactions with the protein. However, the constrained ligands may not be able to adopt the conformation optimal for binding, resulting in lower $E_a$'s. The smaller $E_a$'s measured for the complexes of **3** and **4**, compared with that for **2**, are also consistent with differences in conformational flexibility, as suggested by the kinetic data for the +10 complexes.

In conclusion, we have provided evidence that the bioactive trisaccharide conformer in scFv·**1**$_{(aq)}$ is preserved in the gaseous +10 charge-state complex. The solution conformation of **1** is believed frozen by a network of strong intermolecular H-bonds, some of which are nonspecific in nature, in the gaseous complex. This is, to our knowledge, the first demonstration that aspects of solution conformation in a protein−ligand complex can be preserved in the gas phase. These results are encouraging for the future application of ES-MS and gas-phase techniques to characterize the solution structure of biomolecular complexes.

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**Supporting Information Available:** Experimental procedures, Arrhenius plots for the loss of ligand (L) **1**−**4** from the (scFv + L)$^{11+}$ complexes, and rotamer populations for the Gal-Man linkage in **1**−**4** from MD simulations (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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