J|A|C|S
COMMUNICATIONS

Published on Web 07/20/2002

Evidence for the Preservation of Specific Intermolecular Interactions in Gaseous Protein−Oligosaccharide Complexes

Elena N. Kitova, David R. Bundle, and John S. Klassen*

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

Received February 12, 2002

An attractive, if unproven, strategy to delineate the contribution of solvent effects and intrinsic interactions to the binding affinity of protein−ligand complexes is to compare the structure and stability of the complexes in the gaseous and aqueous phases. Although protein−ligand complexes are readily transferred from solution to the gas phase by nanoflow electrospray ionization (nanoES), it is not yet known to what extent specific intermolecular interactions are preserved in the gas phase. Nonspecific intra- and intermolecular interactions may form during the desolvation process,[1,2] and electrostatic effects, resulting from the presence of multiple charges, may influence structure.[3] Here we provide evidence, based on thermal dissociation experiments combined with functional group replacement, that one of the specific H-bonds within a protein−oligosaccharide complex was preserved in the gas phase.

As there are no direct structural techniques suitable for large gaseous ions, mapping the intermolecular interactions in gaseous protein−ligand complexes represents a considerable experimental challenge. Recently, our laboratory described the application of the blackbody infrared radiative dissociation (BIRD) technique[4] to a series of protonated, gaseous complexes of a single chain variable fragment (scFv) of the monoclonal antibody Se155-4 [5] and its native trisaccharide ligand, Galα[Abe]Man (**1**), and several structural analogues.[2] From a comparison of the Arrhenius activation energies (E_a) measured for the loss of the neutral ligands (S) from the (scFv + S)$^{+10}$ complexes (eq 1),

$$(\text{scFv} + \text{S})^{+10} \rightarrow \text{scFv}^{+10} + \text{S} \qquad (1)$$

the intermolecular interactions originating from the OH groups of the Gal and Man residues (**b** and **c**, Figure 1) were identified and quantified. A significant contribution to the stability of the gaseous complex originated from nonspecific H-bonds at the C-6 OH groups of **b** and **c** which, according to the crystal structure of the scFv·**1** complex,[5] are exposed to solvent. Interactions were also identified at the Gal C-3 and Man C-4 OH groups. In the crystal structure, the Man C-4 OH group forms a direct H-bond with histidine at the 101H position (His^{101H}), while the Gal C-3 OH group does not interact with the protein. However, a direct or water-mediated H-bond between Gal C-3 OH and His^{34L} was identified in crystal structures of the related Fab·**1** complex[6] (H = heavy chain, L = light chain, Fab = antigen-binding fragment of antibody). A comparison of these gas-phase data[2] and the crystal structures suggested that the specific H-bonds originating at the Man C-4 and, possibly, Gal C-3 OH groups may be preserved in the gaseous complex. However, this could not be confirmed in this earlier work because the amino acids that participated in the H-bonds could not be identified.

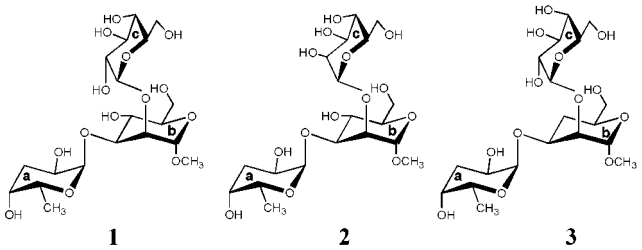

Figure 1. Structures of the trisaccharide ligands. **1** and **2** are C-2 epimers, differing in the orientation of the C-2 OH group of residue **c**; **3** is equivalent to **1** except for the deletion of the Man C-4 OH group.

Here, we report Arrhenius parameters (Table 1) measured for the loss of S from (scFv + S)$^{+10}$ complexes of four mutant scFv's and the trisaccharides: **1**, Talα[Abe]Man (**2**), and Galα[Abe](4-monodeoxy-Man) (**3**), see Figure 1. The mutants differed from the unmodified scFv by the replacement of a single His residue with glutamine (Gln) at the 101H, 34L, 44L, and 97L positions. In the crystal structure, His^{44L} is remote from the binding site and cannot interact with the ligand. This mutant, His^{44L}Gln, served as a control in the present work. His^{97L} is located in the vicinity of the binding pocket but, according to the crystal structure, does not interact with the scFv.

The participation of a given His residue in intermolecular H-bonding was identified by comparing the E_a's measured for the loss of a given ligand from the mutant, in which His was replaced by Gln, and the unmodified scFv. The magnitude of difference in E_a's (i.e., E_a(mutant) − E_a(scFv) = ΔE_a) is expected to reflect the difference in binding of the two side chains (imidazole and propionamide) to the ligand. To our knowledge, accurate energies of binding for these side chains with saccharide OH groups have not been reported. However, interaction energies of 7.9 and 5.5 kcal/mol have been calculated for single H-bonds (of the type −NH--OH$_2$) between water and imidazole(N$_\epsilon$)[7] and *N*-methylacetamide (NMA),[8] respectively. Therefore, replacement of His with Gln is expected to result in a negative ΔE_a, providing Gln engages in a single H-bond. The formation of a bridged structure, with the OH group bonded to both the amide carbonyl and amino groups could result in stronger binding[8] and lead to less negative or even positive ΔE_a's. However, due to conformational constraints within the binding region, a bridging structure is considered unlikely.

For the His^{44L}Gln mutant, a ΔE_a of ∼0 kcal/mol was found for both **1** (−0.1) and **2** (0.0), consistent with there being no interaction between these ligands and His^{44L}. The magnitude of the ΔE_a's also served as a gauge of the precision of the E_a measurements, which we believe is sufficient to identify energetic differences ≥1−2 kcal/mol. Small ΔE_a values were also obtained for the His^{97L}Gln mutant with **1** (−0.5) and **2** (0.1), indicating that His^{97L} did not interact with either of these ligands, in agreement with the crystal structure.

* Corresponding author. E-mail: john.klassen@ualberta.ca.

10.1021/ja025908z CCC: $22.00

Table 1. Arrhenius Parameters Measured for the Reaction (scFv + S)$^{+10}$ → ScFv^{+10} + S, Where S = Trisaccharide (**1**, **2**, and **3**) and scFv = Unmodified and Mutant Single Chain Antibody Fragments

S	scFv	E_a[a] (kcal/mol)	ΔE_a (kcal/mol)	A[a] (s^{-1})
1	**unmodified**	54.3 ± 1.0[c]	-	$10^{27.7 \pm 0.6}$ [c]
1	**His^{44L}Gln**	54.2 ± 0.8	−0.1	$10^{27.7 \pm 0.3}$
1	**His^{97L}Gln**	53.8 ± 0.5	−0.5	$10^{27.4 \pm 0.3}$
1	**His^{34L}Gln**	54.5 ± 0.1	0.2	$10^{27.7 \pm 0.1}$
1	**His^{101H}Gln**	51.3 ± 0.6	−3.0	$10^{26.0 \pm 0.5}$
2	**unmodified**	55.3 ± 1.2[b]	-	$10^{28.7 \pm 0.7}$ [b]
2	**His^{44L}Gln**	55.3 ± 1.3	0.0	$10^{28.4 \pm 0.7}$
2	**His^{97L}Gln**	55.4 ± 0.4	0.1	$10^{28.3 \pm 0.9}$
2	**His^{34L}Gln**	48.9 ± 2.3	−6.4	$10^{24.8 \pm 1.2}$
2	**His^{101H}Gln**	50.0 ± 1.7	−5.3	$10^{25.4 \pm 0.8}$
3	**unmodified**	49.1 ± 1.3[b]	-	$10^{25.4 \pm 0.7}$ [b]
3	**His^{101H}Gln**	49.2 ± 2.2	0.1	$10^{25.3 \pm 1.2}$

[a] Reported errors are one standard deviation. [b] The Arrhenius parameters reported for the unmodified scFv were taken from ref 2. [c] These revised parameters differ from the original values of 55.2 kcal/mol and $10^{28.3}$ s^{-1} reported in ref 2.

For the His^{101H}Gln mutant, a ΔE_a of −3.0 and −5.3 kcal/mol was determined for **1** and **2**, respectively, indicating that the His^{101H} residue interacted with both ligands. According to the crystal structure, His^{101H} is H-bonded to Man C-4 OH. To confirm that this same interaction was present in the gas phase, E_a's were measured for the loss of **3**, in which the C-4 OH group is absent, from the scFv and the His^{101H}Gln mutant complexes. A ΔE_a of 0.1 kcal/mol was determined, indicating that the stabilities of the two complexes were identical in the absence of the C-4 OH group. Therefore, the decrease in E_a measured for **1** and **2** upon replacement of His^{101H} with Gln^{101H} must have resulted from differential binding of these two residues with Man C-4 OH. Taken together, the above results provide compelling evidence that the specific H-bond between His^{101H} and Man C-4 OH was preserved in the gas phase.

Additional insight into the structure of the complexes could be inferred from the aforementioned energetic data. First, the E_a of 49 kcal/mol measured for His^{101H}Gln·**3** is 1−2 kcal/mol smaller than the E_a's measured for complexes of **1** and **2**. This suggests that Gln^{101H} was able to H-bond to **1** and **2**, albeit less strongly than His^{101H}, resulting in the smaller ΔE_a's. Second, the larger ΔE_a measured for **1,** compared with that for **2**, is suggestive of differential binding of the Gln^{101H} residue to the ligands, with a stronger interaction with **1**. From molecular modeling studies performed on complexes of propionamide with **1** and **2**, it was found that the amide group could simultaneously interact with both the Man C-4 and Gal C-2 OH groups. No such bridging structure is possible in the case of propionamide·**2** because the Tal C-2 OH group, which is in the axial position, is too remote to H-bond with the amide group. Therefore, the difference in ΔE_a's is likely due to the ability of the amide side chain of Gln^{101H} to interact simultaneously with the Gal C-2 and Man C-4 OH groups of **1**, while forming a single, weak H-bond to Man C-4 OH in **2**.

For the His^{34L}Gln mutant, a ΔE_a of 0.2 kcal/mol was determined for **1**, suggesting that His^{34L} does not interact with **1**. This result is consistent with the scFv·**1** crystal structure. On the basis of this result, we could further conclude that the interaction originating at Gal C-3 OH, identified in the previous BIRD study[2], must be nonspecific in nature. Surprisingly, a significant ΔE_a (−6 kcal/mol) was determined for **2**, indicating that His^{34L} did interact with this ligand in the gas phase. The only structural difference between **1** and **2** is the configuration of the C-2 OH group of **c** (axial or equitorial). Therefore, the present results suggest that the His^{34L} residue can only H-bond to the C-2 OH group of **c** when it is in the axial position (**2**) and not when it is in the equatorial position (**1**). Consequently, the mutation of the 34L residue results in a significant ΔE_a for **2**, but not for **1**. As there is no crystal structure available for the scFv·**2** complex, it is impossible to comment on whether the H-bond at the C-2 OH group of **2** is present in the condensed phase or whether it forms in the gas phase (i.e. nonspecific). However, it is interesting to note that the association thermochemistry determined by microcalorimetry for **1**[9] and **2**[10] is significantly different, consistent with there being structural differences in the binding region for these two ligands.

The *A*-factors measured for dissociation of these complexes are quite large, $>10^{24}$ s^{-1}. Similar values have been reported for the dissociation of other noncovalent protein complexes.[11] As discussed in detail elsewhere[2], the large A-factors are attributed to the softening of numerous vibrational modes during dissociation of the complex, leading to a large entropy of activation.

In conclusion, we have provided evidence that one of the specific intermolecular scFv·**1** H-bonds was preserved upon transfer of the complex from solution to the gas phase by nanoES. This is, to our knowledge, the first conclusive demonstration that specific protein−ligand interactions can be preserved during the nanoES/desolvation process. The results from this, and the previous BIRD study, indicate that the stability of the gaseous (scFv + **1**)$^{+10}$ complex arises from both specific and nonspecific intermolecular interactions. We anticipate that this behaviour is general for protein−oligosaccharide complexes, although this remains to be confirmed. This work also demonstrates that protein−ligand interactions can be identified and quantified using thermal, gas-phase dissociation experiments. Ultimately, gas-phase methods may play an important role in characterizing ligand interactions with proteins and other biopolymers.

Acknowledgment. We are grateful for financial support provided by the NSERC and an ASMS 2000 Research Award (J.S.K.) and to N. M. Young for generously providing the scFv mutants.

Supporting Information Available: Experimental procedures, Arrhenius plots for the loss of ligand (S) **1**, **2**, and **3** from the (scFv + S)$^{+10}$ complexes, and the intermolecular hydrogen bond map for the scFv·**1** and Fab·**1** complexes (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Wu, Q.; Gao, J.; Joseph-McCarthy, D.; Sigal, G. B.; Bruce, J. E.; Whitesides, G. M.; Smith, R. D. *J. Am. Chem. Soc.* **1997**, *119*, 1157−1158.

(2) Kitova, E. N.; Bundle, D. R.; Klassen, J. S. *J. Am. Chem. Soc.* **2002**, *124*, 5902−5913.

(3) (a) Shelimov, K. B.; Clemmer, D. E.; Hudgins, R. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **1997,** *119*, 2240−2248. (b) Mao, Y.; Woenckhaus, J.; Kolafa, J.; Ratner, M. A.; Jarrold, M. F. *J. Am. Chem. Soc.* **1999,** *121*, 2712−2721.

(4) (a) Tholmann, D.; Tonner, D. S.; McMahon, T. B. *J. Phys. Chem.* **1994**, *98*, 2002−2004. (b) Price, W. D.; Schnier, P. D.; Williams, E. R. *Anal. Chem.* **1996**, *68*, 859−866.

(5) Zdanov, A.; Li, Y.; Bundle, D. R.; Deng, S.-J.; MacKenzie, C. R.; Narang, S. A.; Young, N. M.; Cygler, M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 6423−6427.

(6) Cygler, M.; Bundle, D. R. Unpublished results.

(7) Ornstein, R. L.; Zheng, Y. J. *J. Biomol. Struct. Dyn.* **1997**, *14*, 657−665.

(8) Dixon, D. A.; Dobbs, K. D.; Valentini, J. J. *J. Phys. Chem.* **1994**, *98*, 13435−13439.

(9) Bundle, D. R.; Eichler, E.; Gidney, M. A. J.; Meldal, M.; Ragauskas, A.; Sigurskjold, B. W.; Sinnot, B.; Watson, D. C.; Yaguchi, M.; Young, N. M. *Biochemistry* **1994**, *33*, 5172−5182.

(10) Bundle, D. R. Unpublished results.

(11) Felitsyn, N.; Kitova, E. N.; Klassen, J. S. *Anal. Chem.* **2001**, *73*, 4647−4661.

JA025908Z