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Protein-Ligand Interactions: Probing the Energetics of a Putative Cation- π Interaction

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Abstract

In order to probe the energetics associated with a putative cation- π interaction, thermodynamic parameters are determined for complex formation between the Grb2 SH2 domain and tripeptide derivatives of RCO-pTyr-Ac6c-Asn wherein the R group is varied to include different alkyl, cycloalkyl, and aryl groups. Although an indole ring is reputed to have the strongest interaction with a guanidinium ion ion, binding free energies, G° , for derivatives of RCO-pTyr-Ac6c-Asn bearing cyclohexyl and phenyl groups were slightly *more* favorable than their indolyl analog. Crystallographic analysis of two complexes reveals that test ligands bind in similar poses with the notable exception of the relative orientation and proximity of the phenyl and indolyl rings relative to an arginine residue of the domain. These spatial orientations are consistent with those observed in other cation- π interactions, but there is no net energetic benefit to such an interaction in this biological system. Accordingly, although cation- π interactions are well documented as important noncovalent forces in molecular recognition, the energetics of such interactions may be mitigated by other nonbonded interactions and solvation effects in protein-ligand associations.

One of the most difficult problems in contemporary molecular recognition involving protein-ligand interactions is understanding how and why changes in the structures of small molecules affect relative thermodynamic binding parameters.¹ From a historical perspective, experimental and computational studies for associations of proteins and small molecules typically reported K_i s, IC_{50} s, and binding free energies, G , but since the advent of isothermal titration calorimetry (ITC), binding enthalpies, H , and entropies, S , are more commonly determined.^{2,3} As these data have become available, paradigms to increase ligand potency by modifying ligand structure to enhance binding enthalpies and/or entropies are beginning to emerge.⁴ However, applications of such strategies do not necessarily result in increased affinities because of enthalpy/entropy compensation, which may be virtually

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Supporting Information Available. Methods and materials for ITC and X-ray crystallographic experiments, X-ray diffraction statistics, electron density difference maps, contact diagrams, and plots of thermodynamic data are available free of charge via the Internet at <http://pubs.acs.org>.

balancing,⁵ and because there is often no correlation between G° and either H° or $-T \Delta S^\circ$. Moreover, it is becoming increasingly apparent that some common strategies used to enhance protein binding entropies of small molecules are not uniformly reliable. For example, we discovered that ligand preorganization does not necessarily lead to enhanced protein binding entropies, even when flexible and constrained ligands bind in similar conformations.^{7–9} Although the hydrophobic effect is generally viewed as having a favorable impact on binding entropy,¹⁰ we and others have found that increasing the nonpolar surface area of a ligand can eventuate in more favorable protein binding enthalpies and less favorable binding entropies.^{3,11} Determining the origin(s) of such enthalpy driven hydrophobic interactions is the subject of a number of theoretical studies.¹²

In ongoing studies directed toward correlating structure and energetics in protein-ligand interactions, we recently became interested in explicitly elucidating the energetics associated with cation- π interactions.^{13–18} Such interactions are important structural features in protein folding and protein-ligand interactions and involve a non-covalent interaction between the monopole of a cationic amino group on the side chain of a Lys or Arg residue, and the negatively charged portion of the quadrupole of the aryl group of a Tyr or Trp residue. Although the involvement of such interactions in model systems has been widely studied, there are relatively few investigations directed toward quantifying the detailed energetic contributions of cation- π interactions in protein-ligand associations. For example, Diederich has shown that cation- π interactions contribute about 2.8 kcal mol⁻¹ to binding free energy for complexation of ligands to the aromatic box of factor Xa, but binding enthalpies and entropies were not reported.^{16a,b} On the other hand, Marshall and coworkers have found that such interactions can be mitigated by competing, adjacent salt-bridges.¹⁸

We previously identified the SH2 domain of the growth receptor binding protein 2 (Grb2), a cytosolic adapter protein that participates in the Ras signal transduction pathway,¹⁹ as an excellent model system for studying molecular recognition in a biological system.^{8,11c} In the context of cation- π interactions, Furet and coworkers discovered that the affinity of **3** (IC₅₀ = 65 nM) for the Grb2 SH2 domain was about two orders of magnitude greater than for the related tripeptides **1** and **2**, which were approximately equipotent.²⁰ Based upon modeling studies, they attributed the enhanced potency to favorable stacking, or a cation- π interaction, between the electron-rich aniline ring at the *N*-terminus of **3** and the Arg67 residue of the Grb2 SH2 domain. In a subsequent study, Nioche and coworkers found that the related phosphotyrosine derivatives **4–6** bound with approximately equal affinity (IC₅₀ of **6** = 13 nM) to the Grb2 SH2 domain.²¹ An X-ray study revealed that the aromatic ring of the *m*-amino-Cbz group of **6** in its complex with the domain aligned in a parallel, but not completely stacked, orientation relative to the plane of the Arg67 residue.

In order to probe the role and detailed energetics associated with the putative cation- π interactions between the Grb2 SH2 domain and tripeptide ligands related to **3** and **6**, the phosphotyrosine analogs **7–12** were prepared, and the thermodynamic binding parameters (K_a , G° , H° , S°) for their associations with the Grb2 SH2 domain were determined by isothermal titration calorimetry (ITC) (Table 1). The selection of the Ac6c replacement for the pTyr+1 residue was predicated upon the requirement that we wanted a series of high affinity compounds, and the known compound **9**²² ($IC_{50} = 1$ nM) was about 65-fold more potent than **3** and comparable in potency to **4–6**. Compounds **7–9** were prepared for comparison with compounds **1–3** and **4–6** of the prior art. The indole analog **10** was selected because the indole side-chain of Trp is the aromatic group most commonly involved in energetically significant cation- π interactions in proteins.^{14c} Compound **11** would enable a comparison with the carbamate **8** and the cyclohexane analog **12** would be a control for **11**.

Examination of the ITC data in Table 1 reveals that **7–9** bind to the Grb2 SH2 domain with approximately equal affinity, an observation consistent with the findings of Nioche²¹ but not Furet.²⁰ The chain linking the carbonyl and phenyl groups of **8** has an oxygen atom, whereas there are only carbon atoms in the linking chain of **11**. This variation in atom types may result in subtle conformational or desolvation effects that account for the slightly more favorable binding free energy of **11**, the origin of which appears to be entropic. Furthermore, in contrast to what we expected based upon a putative cation- π interaction, the phenyl and cyclohexyl derivatives **11** and **12**, respectively, were about two-fold more potent than the corresponding indolyl compound **10**. Given the narrow range of G° values for **10–12** and experimental error, interpreting the importance of the minor differences in corresponding H° and $-T \Delta S^\circ$ values is problematic. However, given the close similarities in the thermodynamic parameters for **10–12**, a cation- π interaction does not appear to contribute significantly to relative binding energetics.

Although we sought to analyze the structures of the complexes of **8–12** with the Grb2 SH2 domain, suitable crystals were only obtained for complexes of **8** and **10**. These structures

were solved at resolutions of 1.6 and 1.8 Å, respectively, by molecular replacement using the structure of the domain in complex with the parent molecule **7** (PDB code: 3S8O).²³ Alignment of the backbone atoms belonging to the domain in the complexes of **8** and **10** yields root mean square deviations (RMSDs) for all backbone atoms of <0.2 Å (Figure 1). Although there are obvious differences in the spatial orientations of the pTyr-1 side-chains in the bound forms of **8** and **10**, there is little variation in the positions of equivalent atoms of **8** and **10**, which superimpose with a RMSD < 0.2 Å, which is less than the coordinate error that is associated with the molecular models,²⁴ following alignment of the domains.

In their respective complexes, both **8** and **10** make 14 direct and five water-mediated polar contacts to the domain.²⁵ The cyclohexane ring at the pTyr+1 position in each complex makes a total of 15 van der Waals (vdW) contacts with the domain.²⁶ The favorable nonbonded interactions between the Grb2 SH2 domain and the atoms common to both **8** and **10** are thus virtually identical to one another. However, because the phenyl group of **8** is positioned closer to Arg67 of the domain than the indolyl moiety of **10**, **8** makes 23 vdW contacts with the pTyr-1 subsite, whereas **10** makes only five such contacts. However, this large difference in the number of vdW contacts is not reflected in any significant differences in the thermodynamic binding parameters (Table 1).

Cation- π interactions involving arginine side chains in proteins tend to adopt a so-called parallel electron donor-acceptor geometry rather than an oblique or T-shaped geometry.^{14c,15} Examination of the structure of the complex of **8** with the domain reveals that the phenyl ring in the Cbz group indeed adopts such a parallel geometry relative to the guanidinium ion of Arg67 (Figure 2a), and the carbon atoms of the phenyl ring are 3.4–3.8 Å removed from the central carbon atom of guanidinium group. On the other hand, the distance between the carbon atoms in the benzenoid ring of the indole ring and the nearest nitrogen atom of the guanidinium group in the complex of **10** range from 3.5–4.5 Å, and the planes of the guanidinium moiety of Arg67 and the indole ring at the pTyr-1 site of **10** are oblique (Figure 2b). Although this is not the preferred relative orientation in protein structures, it is not possible to confidently correlate variations of energetics with interplane angle, because there are strong cation- π interactions having oblique geometries.^{14c}

Comparing the results of these studies with those of Furet²⁰ and Nioche²¹ clearly shows that energetic effects associated with varying the pTyr-1 side chain of Grb2 SH2 binding ligands depend upon the nature of the pTyr+1 residue. X-Ray crystallographic studies of **8** and **10** reveal that the relative orientations of the guanidine moiety of Arg67 and the aromatic groups in the side chains of **8** and **10** are consistent with what is expected for a cation- π interaction, although the geometry in the complex of **10** is not ideal. If one assumes that the binding poses for **8**, **11**, and **12** are similar, any net contribution of a cation- π interaction to the binding free energies in this system are negligible. Indeed, the observation that *both* phenyl and cyclohexyl group replacements of an indole ring give ligands of comparable potency indicates that other factors are at play. Solvation effects may be a play, but interactions of other functional groups on the ligand with Arg67 may be more significant. For example, Marshall has suggested that energetics associated with adjacent salt-bridges may dominate cation- π interactions.¹⁸ Accordingly, the salt-bridge between Arg67 and the phosphate groups of **8** and **10**, which have interaction distances of 2.8 Å (Figure 2), may

mitigate the energetic effects of any cation- π interaction. There are hydrogen bonding interactions, which range from 2.8–3.3 Å, between the pTyr-1 carbonyl oxygen atoms and the two N η atoms of the Arg67 side chains in these complexes. Thus, although cation- π interactions are well documented as important noncovalent forces in molecular recognition, the energetics of such interactions may be mitigated by other nonbonded interactions and solvation effects in protein-ligand associations.

Supplementary Material

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26. A vdW contact is defined by an interatomic distance in the range of 3.4–4.2 Å between a carbon atom of the bound ligand and a carbon, nitrogen, or oxygen atom in the domain.

Figure 1. Complexes of the Grb2 SH2 domain with **8** and **10**. Oxygen, nitrogen, and phosphorous atoms are colored red, blue, and orange, respectively. Carbon atoms belonging to the complex with **8** and **10** are colored magenta and cyan, respectively. (a) Alignment of backbone atoms belonging to the domains in the complexes, showing the domains (ribbons) and the bound ligands (sticks). (b) The bound ligands (sticks) only. Dashed black line represents the internal hydrogen bond between the pTyr carbonyl oxygen atoms and the amide nitrogen atoms of the pTyr+3 sites.

Figure 2. Interactions between the pTyr-1 and pTyr moieties of the bound ligands (sticks) in the complexes of **8** (a) and **10** (b) and Arg67 of the Grb2 SH2 domain (lines); interactions between N η atoms of Arg67 and the phosphate and pTyr-1 C=O groups not shown (see text).

Table 1

Thermodynamic data for complexes of **7–12** and the Grb2 SH2 domain. ^[a,b]

Ligand	R	K_a ($\times 10^6 \text{ M}^{-1}$)	G° (kcal mol^{-1})	H° (kcal mol^{-1})	$-T S^\circ$ (kcal mol^{-1})
7	Me	7.0 ± 1.2	-9.3 ± 0.1	-8.5 ± 0.4	-0.8 ± 0.4
8		6.5 ± 0.1	-9.3 ± 0.1	-9.3 ± 0.3	0.0 ± 0.1
9		8.8 ± 0.9	-9.5 ± 0.1	-9.1 ± 0.5	-0.4 ± 0.3
10		11.4 ± 0.3	-9.6 ± 0.1	-10.0 ± 0.3	$+0.4 \pm 0.1$
11		23.4 ± 0.9	-10.1 ± 0.1	-9.5 ± 0.5	-0.6 ± 0.1
12		28.8 ± 1.3	-10.2 ± 0.1	-10.0 ± 0.5	-0.2 ± 0.1

^[a]ITC experiments were conducted at 25 °C in HEPES (50 mM) with NaCl (150 mM) at pH 7.45 $\pm$ 0.05 as previously reported.^{8b}

^[b]Three or more experiments were performed for each ligand, and the averages are reported following normalization of the n values for each experiment by adjusting ligand concentration (See Supporting Information). Errors in the thermodynamic values were determined by the method of Krishnamurthy.^{2a}