Synthetic Adenine Receptors: Direct Calculation of Binding Affinity and Entropy

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Abstract: A novel method for calculating binding free energies is applied to a series of water-soluble adenine receptors that have been characterized experimentally. The calculations use a predominant states method, "Mining Minima", to identify and account for the low-energy conformations of the free and bound species. The CHARMM force field is used to estimate potential energies, and an adjusted form of the generalized Born/surface area model is used to estimate solvation energies as a function of conformation. The computed binding free energies agree with experiment to within 2.9 kJ/mol (0.7 kcal/mol) and reproduce observed trends across the series of receptors. Preorganization of two rotatable bonds enhances the calculated affinity of one receptor/adenine complex by −2.5 kJ/mol (−0.6 kcal/mol), and the change in translational/rotational entropy ($-T\Delta S^{\circ}_{trans/rot}$) is 30 kJ/mol (7 kcal/mol). The concept of the translational/rotational entropy change upon binding in the present model is compared with others previously presented in the literature.

Introduction

Although biomacromolecules present arguably the most impressive examples of molecular recognition, smaller host−guest systems are of great interest as well. The association of nonmacromolecules has practical applications in a number of areas, including chemical separations, catalysis, and drug delivery. Because of their relative simplicity, host−guest systems are also useful as test cases for computer models of binding. In tests on macromolecules, the adequacy of the conformational sampling in the calculations is often in question, so the success or failure of a calculation may not reflect the validity of the underlying theory and energy model. In contrast, small molecule systems may be simple enough to permit all relevant molecular conformations to be identified and accounted for in the calculations.

We have recently developed an efficient method for the direct calculation of free energies and binding affinities[1,2] that has given promising results for a series of simple systems.[3−8] This method is applied here to seven synthetic adenine receptors that were developed to explore nucleic acid base-pairing in water.[9,10]

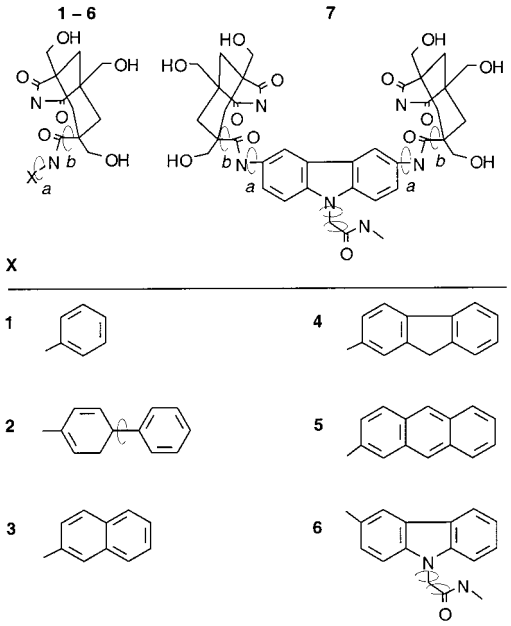

Figure 1. Seven synthetic adenine receptors. The ovals represent the rotatable bonds discussed in the text.

The receptors are equipped with imide moieties that can form hydrogen bonds with adenine and with a variety of different aromatic groups that permit stacking (Figure 1). The calculations yield standard free energies of binding (absolute binding free energies[11]), permitting direct comparison with measured binding affinities. The calculations also allow issues of preorganization and entropy to be addressed in the context of well-defined theory. Thus, the influence of rigidifying rotatable bonds on

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the binding affinity is examined, and changes in translational/rotational entropy are calculated and compared with values from previous publications. The relationship of the present definition the translational/rotational entropy change on binding is related to the Sackur−Tetrode equation[12,13] and the concept of "cratic" entropy.[14−17]

Methods

Calculation of Binding Free Energy. The "Mining Minima" (MM) algorithm is used to compute the aqueous binding affinities of adenine with the seven receptors illustrated in Figure 1. Detailed descriptions of the algorithm and its use have been presented elsewhere.[2,4,5] Briefly, the standard free energy change ΔG° when receptor R and ligand L form the noncovalent complex $R{\cdot}L$ is given by[18]

$$\Delta G^\circ = -RT \ln\frac{Z_{R\cdot L}}{Z_R Z_L} + RT \ln\frac{8\pi^2}{C^\circ} \qquad (1)$$

where Z_X is the configuration integral of the subscripted species, C° is the standard concentration (typically 1 mol/L), and RT is thermal energy. The second term derives from the three external rotations and translations that are converted into internal degrees of freedom in the $R{\cdot}L$ complex. The configuration integrals are of the form

$$Z_X \equiv \int \exp(-E(\mathbf{r})/kT)\mathrm{d}\mathbf{r} \qquad (2)$$

where $E(\mathbf{r})$ is the energy as a function of the conformation $\mathbf{r}$ and kT is thermal energy. As discussed later, the energy $E(\mathbf{r})$ is computed as a sum of potential and solvation energy.

The MM algorithm computes the configuration integrals Z_X by identifying each low-energy conformation i and evaluating the configuration integral $Z_{X,i}$ for the corresponding energy well via a Monte Carlo method. The complete configurational integral of species X is then

$$Z_X \approx \sum_i Z_{X,i} \qquad (3)$$

The sum over energy minima is extended until the Boltzmann-averaged energy $\langle E(\mathbf{r})\rangle$ converges to within a predefined tolerance, as described previously.[5]

Conformational Sampling. Initial all-atom coordinates for adenine and the receptors were generated with Quanta 97.[19] Each molecule was then energy-minimized in an appropriate conformation (vide infra) with the full CHARMM 98 vacuum energy function by the Newton−Raphson method[20] in version 26 of CHARMM.[21] The minimizations were terminated when the energy gradient changed by $<4.0 \times 10^{-5}$ kJ/mol-Å per step. Conformational sampling for the free receptors extended over their key rotatable bond, as detailed later. For the bound complexes, sampling also extended over the 6 positional and orientational degrees of freedom of adenine relative to each receptor.

Experimentally, the association of adenine with the receptors was detected by monitoring the NMR chemical shifts of exchangeable protons[9] that form receptor−ligand hydrogen bonds in the complex.

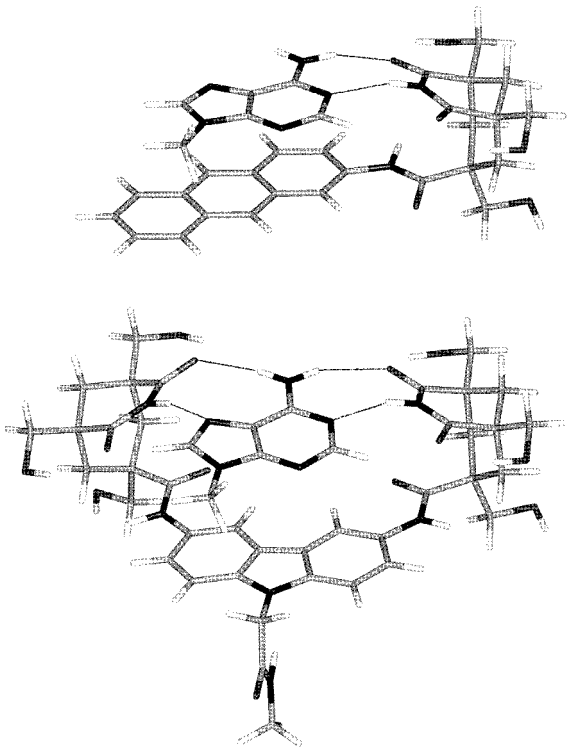

Figure 2. Stable adenine binding modes for mono-imide receptor **5** (top) and bis-imide receptor **7** (bottom).

Therefore, complexes without hydrogen bonds were not detected. To match experimental conditions, conformations of the complexes with no receptor−ligand hydrogen bonds were omitted from the configuration integrals of the complexes. This was accomplished by omitting those conformations in which adenine and the imide group were on opposite sides of the aromatic stacking surface.

For simplicity, the two hydroxyl groups near the imide carbonyls were kept fixed in conformations in which they form internal hydrogen bonds with the carbonyls (Figure 2). The error from this restriction is small, as evidenced by test calculations for receptor **5** in which binding affinities were calculated with the complex in its four most stable conformations and with the hydroxyls either fixed or mobile; the change in the computed binding free energy upon mobilizing the hydroxyls was <0.4 kJ/mol.

It is possible to save computer time by exploiting the internal symmetries of the receptors. For example, a torsion that controls the rotation of a simple phenyl group can be sampled over only $[0,\pi]$ rather than the full $[0,2\pi]$ range as long as this sampling is done consistently in both the free and bound species. In some cases, reflection symmetries also can be exploited.

For receptors **1** and **2**, the aryl bond (a) is sampled over $[0,\pi]$ instead of $[0,2\pi]$ to take advantage of the 2-fold rotational symmetry of the aryl group. Similarly, the biphenyl bond in **2** is sampled from $[0,\pi]$. The imide bond (b) is sampled over $[0,\pi]$, based on the following reasoning. Rotation by π for the free receptor generates a collection of mirror image conformations whose energy is the same as that with the unrotated imide bond. Therefore, the configuration integral Z_R can be computed by sampling over only $[0,\pi]$, and then multiplying by 2 to correct for the mirror-image conformations that are thus neglected. Because adenine is not chiral, its interactions with the reflected receptor are the same as those with the unreflected receptor. Therefore, it is again correct to sample the imide bond over $[0,\pi]$ for the complex and to multiply the resulting configuration integral, $Z_{R\cdot L}$, by 2 to correct for the neglected conformations. Note that the factors of 2 for Z_R and $Z_{R\cdot L}$ cancel in eq 1.

For receptors **3**, **4**, and **5**, there is no rotational symmetry at the aryl bond (a) of these compounds, so this bond is sampled over the range $[0,2\pi]$. The most stable positions of these aryl rotations are those in which the aryl groups are coplanar with the amides. Because the aryl

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groups are asymmetric, there are two such conformations, separated by π radians. To make sure that both of these conformations are thoroughly sampled, the configuration integrals were carried out in two parts. In each part, the bond lengths and angles were optimized with one or the other of the stable aryl orientations and the aryl bond was allowed to vary over only the corresponding half of its 2π range. The imide bond (b) in these compounds is sampled over only $[0,\pi]$, based on the same reasoning applied to receptors **1** and **2**.

The N-linked amide "tail" of receptor **6** could, in principle, adopt a range of conformations. However, test calculations showed that this group occupies only the two conformers in which the NH_2 group forms a hydrogen bond with the ring nitrogen from either the top or the bottom of the ring system. We therefore fixed the tail rigidly in one of these conformers for both receptors. This conformation results in a receptor whose symmetry is identical to that of receptors **3**, **4**, and **5**, so the same sampling protocol is used.

For the free receptor **7**, rotating the two aryl bonds or the two imide bonds by π radians produces distinct conformations, so all four bonds are sampled over their full 2π range. To ensure complete sampling, the integration over each aryl and imide bond was carried out in two segments of π radians, with the bond lengths and angles optimized for the particular range, for a total of 16 integrals. (In the end, however, the results proved insensitive to the starting conformation.) As in the case of **6**, the amide tail was fixed in one of its optimal position in both the free and bound receptor. For the complex of **7** with adenine, the four conformations with both imide moieties on the side of the aryl group away from the amide tail are ~16 kJ/mol (3.8 kcal/mol) more stable than the other 12 possibilities. This stability allowed the configuration integral of the complex to be simplified by including only these four contributions.

Energy Model. The energy in the configuration integrals can be separated into a potential energy U and a solvation energy W:[18] $E(\mathbf{r}) = U(\mathbf{r}) + W(\mathbf{r})$. Here, the potential energy was computed with the all-atom CHARMM parameter set as of May 1998 (A. D. MacKerell, personal communication),[21−23] except that equilibrium bond lengths and angles for the amide−aryl linkages were obtained via ab initio quantum mechanical optimizations performed at the HF/6-31G* level with the program GAMESS.[24] The molecular dielectric constant was set to 1 as specified in the force field parametrization.[22,23]

The solvation energy was estimated with the generalized Born model (GB)[25−27] during sampling, and further adjusted at each energy minimum as described later. The solvent dielectric constant was set to that of water (84 at 10 °C), and the cavity radius of each atom was set to the mean of the solvent probe radius and the atom's σ parameter from the Lennard−Jones part of the CHARMM force field.[3] The radius of the water solvent probe was taken to be 1.4 Å. The nonpolar component of the solvation energy was estimated as proportional to the solvent-accessible surface area, with a proportionality constant of 26.75 J/mol Å^2.[28]

The GB model was adjusted as follows to optimize the agreement with more accurate finite difference (FD)[29−31] solutions of the Poisson equation obtained with the program UHBD.[32] For each of the distinct local minima identified during conformational sampling, the electrostatic solvation energy was recalculated with both GB and FD. The deviation of GB relative to FD of the minimum was then subtracted from the original conformational free energy of the minimum. Previous studies indicate that this procedure improves the agreement of computation with experiment.[5] The surface area contribution to the free energy of each energy minimum was included in a similar manner.[5]

Table 1. Computed and Experimental Standard Free Energies of Binding[a]

receptor	ΔG°_{comp}	ΔG°_{expt}
1	−2.1 (−0.5)	−1.7 (−0.4)
2	−4.6 (−1.1)	−6.3 (−1.5)
3	−5.4 (−1.3)	−7.9 (−1.9)
4	−6.3 (−1.5)	−9.2 (−2.2)
5	−7.9 (−1.9)	−10.0 (−2.4)
6	−6.7 (−1.6)	−8.4 (−2.0)
7	−8.8 (−2.1)	−9.6 (−2.3)

[a] Results are in in kJ/mol (kcal/mol) in water at 10 °C. The standard state is a hypothetical ideal 1 mol/L aqueous solution.

Results and Discussion

Binding Affinities. As shown in Table 1, the calculated binding free energies are in the correct range, indicating that the model appropriately balances the contributions of potential energy, solvation energy, and solute entropy. Indeed, the individual calculations agree with experiment to within a maximal error of 2.9 kJ/mol (0.7 kcal/mol). In addition, the calculations reproduce the observed increase of affinity with increasing size of the aromatic stacking surface for the monoimide receptors, although the strength of this trend is somewhat underestimated by the calculations. The overall agreement with experiment found here supports the validity of the present approach to computing binding affinities.

The present calculations are reasonably fast: one receptor−ligand calculation, which accounts for a large number of potential binding modes, takes ~1−2 days on a fast workstation, and work in progress suggests that greater efficiency can be attained. It would be much more difficult to address these systems with double-annihilation or related methods,[11,18,33−35] especially if an explicit model of the solvent were used. Such methods typically require the user to identify the major distinct binding modes in advance of the calculation. The present method, in contrast, automatically identifies and accounts for the most stable conformations of the complex. This automatic identification avoids the need for the user to make an arbitrary selection of the important binding modes in setting up the calculation.

Binding Modes. The receptors were designed to interact with adenine via hydrogen bonding to the imide group(s) combined with hydrophobic stacking on the aromatic groups.[9,10] The low-energy bound conformations identified here are consistent with this design, as illustrated by the low-energy conformations shown in Figure 2. It was also expected that the monoimide complexes would adopt Watson−Crick, reverse Watson−Crick, Hoogsteen, and reverse Hoogsteen hydrogen-bonding patterns.[9,10] The calculations are consistent with this expectation as well, because they ascribe similar stabilities to these four

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forms in the case of the monoimide receptors. For example, the computed free energies of these conformations lie within a 1 kJ/mol (0.2 kcal/mol) range for receptor **5**. On the other hand, we find a single dominant hydrogen-bonding pattern for the bis-imide complex (Figure 2, bottom).

Preorganization and Entropy. The entropy changes associated with noncovalent binding are subject to continuing interest and debate, as recently reviewed.[18] The computational method described here allows this issue to be examined quantitatively.

First, the influence of preorganization was examined by computing the increment in binding free energy that results from rigidifying receptor **5** in the conformation it adopts in the global energy minimum for its complex with adenine. When the binding calculation is repeated with the two rotatable bonds (a and b in Figure 1) locked in this optimal conformation, the standard binding free energy falls from −7.9 to −10.5 kJ/mol (−1.9 to −2.5 kcal/mol). Interestingly, this 2.5 kJ/mol (0.6 kcal/mol) change matches an empirically derived value of 1.25 kJ/mol (0.3 kcal/mol) for the enhancement in binding energy upon rigidifying a single rotatable bond.[36] However, this quantity is likely to be case dependent: rigidifying a rotatable bond that is not constrained upon binding is not expected to promote binding, all other things being equal. Also, a chemical modification that rigidifies a bond will not produce the anticipated improvement in binding if the modification has effects in addition to preorganization.

For a rigid receptor and a rigid ligand, the only changes in entropy upon binding are those that result from the reduction in translational and rotational freedom of the molecules and from changes in the entropy of the solvent. Hence, the change in translational and rotational entropy can be obtained by computing the entropy change while treating the solvation energy as a temperature-independent potential energy rather than a temperature-dependent free energy;[18] thus

$$-T\Delta S^\circ_{\text{trans/rot}} = \Delta G^\circ - (\langle U + W\rangle_{\text{bound}} - \langle U + W\rangle_{\text{free}}) \quad (4)$$

Here the angle brackets indicate Boltzmann-weighted averages for the bound and free states as indicated. These quantities are readily computed in the course of the MM calculations. The resulting value of $-T\Delta S^\circ$ for the association of adenine with the rigidified receptor **5** is +29.7 kJ/mol (7.1 kcal/mol) at 283 K. This value is similar to that calculated for the association of benzene with a mutant T4 lysozyme[35] [+35.1 kJ/mol (8.4 kcal/mol) at 300 K].

Note that the change in translational/rotational entropy value upon binding as defined here is not a universal constant, but depends on the relative mobility of the two complexed molecules. Indeed, the calculated value of $-T\Delta S^\circ_{\text{trans/rot}}$ for the association of guanidium and phosphate ions in water is only 5.7 kJ/mol (1.3 kcal/mol),[5] indicating considerable mobility within the complex. This mobility results in part from the fact that the complex can adopt eight distinct conformations that are essential equi-energetic.[5] In addition, each of these conformations may retain considerable mobility because there are only two points of contact between the molecules, and because binding is mediated largely by long-ranged electrostatic interactions that allow considerable play in the structure.

The changes in translational/rotational entropy reported here are independent of molecular mass, consistent with our use of the classical approximation to statistical thermodynamics.[12,18,37] The appearance of molecular mass in the classical Sackur−Tetrode equation for translational entropy is sometimes taken to imply that the translational entropy change on binding does depend on mass (see, for example, refs 13, 38, and 39). However, the concept of a mass-dependent entropy change on binding is ambiguous because the masses of the two molecules that bind are often different and there is no basis for choosing one mass over the other. The fact is that when the overall entropy of binding is computed with the Sackur−Tetrode equation along with the classical expressions for rotational and vibrational entropy, the result is independent of mass, a point that will be elaborated elsewhere.

The idea of a mixing or "cratic" entropy also has been used to account for losses in translational entropy upon binding.[14−17] It would be of interest to compare our results with those obtained from the cratic model. However, we are not aware of any prescription for treating losses in rotational entropy within the cratic formulation, so a direct comparison would not be appropriate. What can be said is that the entropy changes considered in the present work have rather different properties from the cratic entropy as defined by Gurney.[14] For one thing, changes in translational and/or rotational entropy upon binding do not appear as distinct terms in the present formulation. Rather, they are implicit in the overall free energies and can be extracted only through additional computational steps. In contrast, the cratic entropy appears as a distinct term in calculations of binding free energies. Moreover, the cratic entropy of binding is the same for every pair of molecules, and depends only on the standard concentration and the concentration of the solvent (55 mol/L for water). In contrast, the losses in translational/rotational entropy calculated here increase as the rigidity of the complex increases, as might be intuitively expected for this quantity. Amzel has recently proposed a derivation of the cratic entropy based on a cell theory of liquids.[17] Perhaps this approach could be extended to identify the relationship between the cratic concept and the theory used in the present work.

Conclusion

We demonstrate that the binding free energy of aqueous receptor−ligand systems can be computed with good accuracy via a direct algorithm that automatically identifies and accounts for the most stable conformations of the free and bound species. The calculations rely on an empirical force field and an implicit solvation model based on continuum electrostatics. The agreement with experiment supports the validity of the energy model and of the underlying theory. It also allows issues of configurational and translational/rotational entropy to be addressed quantitatively within the context of a well-defined theoretical framework.

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