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On the calculation of binding free energies using continuum methods: Application to MHC class I protein–peptide interactions

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Abstract

This paper describes a methodology to calculate the binding free energy (ΔG) of a protein-ligand complex using a continuum model of the solvent. A formal thermodynamic cycle is used to decompose the binding free energy into electrostatic and non-electrostatic contributions. In this cycle, the reactants are discharged in water, associated as purely nonpolar entities, and the final complex is then recharged. The total electrostatic free energies of the protein, the ligand, and the complex in water are calculated with the finite difference Poisson-Boltzmann (FDPB) method. The nonpolar (hydrophobic) binding free energy is calculated using a free energy-surface area relationship, with a single alkane/water surface tension coefficient (γ_{aw}). The loss in backbone and side-chain configurational entropy upon binding is estimated and added to the electrostatic and the nonpolar components of ΔG. The methodology is applied to the binding of the murine MHC class I protein H-2K^b with three distinct peptides, and to the human MHC class I protein HLA-A2 in complex with five different peptides. Despite significant differences in the amino acid sequences of the different peptides, the experimental binding free energy differences ($\Delta\Delta G_{exp}$) are quite small (<0.3 and <2.7 kcal/mol for the H-2K^b and HLA-A2 complexes, respectively). For each protein, the calculations are successful in reproducing a fairly small range of values for $\Delta\Delta G_{calc}$ (<4.4 and <5.2 kcal/mol, respectively) although the relative peptide binding affinities of H-2K^b and HLA-A2 are not reproduced. For all protein–peptide complexes that were treated, it was found that electrostatic interactions oppose binding whereas nonpolar interactions drive complex formation. The two types of interactions appear to be correlated in that larger nonpolar contributions to binding are generally opposed by increased electrostatic contributions favoring dissociation. The factors that drive the binding of peptides to MHC proteins are discussed in light of our results.

Keywords: binding free energy; hydrophobic effect; Major Histocompatibility Complex; Poisson-Boltzmann electrostatics; protein–peptide interactions; solvation free energy

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Abbreviations and symbols: FDPB method, finite difference Poisson-Boltzmann method; MHC, Major Histocompatibility Complex; PDB, Brookhaven Protein Data Bank; RMS, root mean square; K_D, equilibrium dissociation constant; ΔG_{exp}, experimental binding free energy; ΔG_b, theoretical binding free energy; ΔG_{calc}, calculated binding free energy; ΔG_{coul}, Coulomb contribution to binding; ΔG_{solv}, reaction field (solvation) contribution to binding; ΔG_{el}, electrostatic contribution to binding (sum of ΔG_{coul} and ΔG_{solv}); ΔG_{np}, nonpolar (hydrophobic) contribution to binding; ΔG_{strain}, change in conformational free energy of both the receptor and the ligand upon binding; ΔS_{mc} and ΔS_{sc}, loss of configurational entropy due to the freezing of backbone and side-chain torsional angles upon binding; $\Delta S_{t,r}$, loss of translational and rotational degrees of freedom upon binding; A, solvent-accessible surface area; γ_{aw}, microscopic surface tension associated with the transfer of alkane from liquid alkane to water; ϵ_o, dielectric constant of water; ϵ_i, dielectric constant of macromolecular interior.

The accurate calculation of ligand-protein binding free energies is a difficult problem for which no general solution has been forthcoming. Molecular dynamics simulations including the explicit treatment of solvent molecules have encountered a number of striking successes (see, e.g., Bash et al., 1987; McCammon, 1987; Beveridge & DiCapua, 1989; Straatsma & McCammon, 1991; Miyamoto & Kollman, 1993; Kollman, 1993, 1994); however, the need to sample a large ensemble of conformational states places severe computational demands on this approach. At the other extreme, a variety of empirical methods have also been developed to estimate binding free energies (see, e.g., Andrews et al., 1984; Williams et al., 1991, 1993; Böhm, 1994). These methods, which, for example, assign a free energy value to each hydrogen bond, salt bridge, and buried nonpolar area, provide extremely useful qualitative tools. However, they are not

generally able to yield accurate quantitative results (Ajay & Murcko, 1995).

An intermediate approach involves the use of continuum methods to describe solvent molecules while retaining an atomic level description of the protein. Continuum methods have been applied with some successes to the calculation of relative binding free energies for closely related ligands (Wendoloski et al., 1993; Jackson & Sternberg, 1995; Shen & Quiocho, 1995; Shen & Wendoloski, 1995; Zhang & Koshland, 1996). In this paper we describe a general method for the calculation of binding free energies using continuum solvent methods and apply this method to the study of peptide-protein interactions in MHC class I protein–peptide complexes. Specifically, we consider the binding of the murine MHC class I protein, H-2K^b, with three distinct peptides and of the human MHC class I protein, HLA-A2, with five different peptides (see Table 1 for experimental details about each complex). Despite significant differences in the sequences of the peptides binding to each protein, the observed binding free energies are quite close. Reproducing this result provides a significant challenge to any theoretical methodology.

The structural diversity of the peptidic ligands to MHC proteins is enormous; about 10% of all possible peptide sequences of appropriate length may bind to the same MHC class I protein with nanomolar affinities (Ruppert et al., 1993). Understanding the structural and energetic basis of this combination of low specificity and high affinity is a problem of great biophysical as well as biological relevance. Moreover, in addition to their intrinsic interest, MHC class I protein–peptide complexes provide an ideal test system for theoretical methods since there is a great deal of both structural and thermodynamic data available for an extremely diverse set of ligands.

Our approach is based on the use of the finite-difference Poisson-Boltzmann (FDPB) method (Warwicker & Watson, 1982; Nicholls & Honig, 1991) to calculate electrostatic interactions, and a free energy-surface area relationship to estimate nonpolar contributions to binding. We use PARSE parameters that are known to yield good solvation free energies for peptidic systems (Sitkoff et al., 1994) and that yield hydrogen-bonding energies that are similar to those obtained from high level quantum mechanical calculations (Ben-Tal et al., 1997). Thus, all parameters used in our calculations have been determined by fitting to an independent data set (see below). The level of agreement between theory and experiment that is obtained here suggests that the underlying physical model describes many of the important elements of the binding reaction. On the other hand our study also highlights a number of the shortcomings in the methodology as will be discussed in some detail in the Discussion.

Results

Precision of the calculations

In order to assess the precision of the finite difference Poisson-Boltzmann calculations, we tested the sensitivity of the calculated value of ΔG_{solv} (265.2 kcal/mol for H-2K^b/VSV-8; Table 2) to the rotation and translation of the coordinates of the H-2K^b/VSV-8 complex within the lattice used in the finite difference calculations. Twenty-seven positions obtained by rotating the complex in 60° increments around the *x*, *y*, and *z* axes were generated. The computed value of ΔG_{solv} had a standard deviation of only 0.75 kcal/mol. The center of coordinates of the H-2K^b/VSV-8 complex was also translated in the lattice in 0.2 Å increments along the *x*, *y*, *z*, *xy*, *xz*, and *yz* axes. In this case, the standard deviation of ΔG_{solv} was only 0.13 kcal/mol.

Dependence on atomic coordinates

We tested the sensitivity of the calculations to the structural model used by carrying out binding energy calculations on both the crystal structure (with hydrogen atom positions generated as described in Methods), and on energy-minimized structures of the H-2K^b/VSV-8 complex. The binding free energy of the complex (ΔG_{calc}) is −22.3 kcal/mol using the crystal structure, −36.8 kcal/mol for the structure minimized without restraints, and −26.4 kcal/mol for

Table 1. *Protein–peptide complexes used in analysis*

MHC class I protein	Peptide source	Peptide position	Peptide sequence	Peptide abbreviation	PDB file[a]	ΔA^b (Å^2)	K_D[c] (nM)	ΔG_{exp}[d] (kcal/mol)
H-2K^b	Sendai virus nucleoprotein	324–332	FAPGNYPAL	SEV-9	1VAB[e]	−1,695	2.7[f]	−11.7
H-2K^b	Vesicular stomatitis virus nucleoprotein	52–59	RGYVYQGL	VSV-8	1VAA[e]	−1,857	3.7[f]	−11.5
H-2K^b	Chicken ovalbumin	257–264	SIINFEKL	OVA-8	1VAC[g]	−1,807	4.1[f]	−11.4
HLA-A2	Hepatitis B virus nucleocapsid	18–27	FLPSDFFPSV	HBV-10	1HHH[h]	−1,926	3.3[i,j]	−11.6
HLA-A2	Influenza A virus matrix M1	58–66	GILGFVFTL	FLU-9	1HHI[h]	−1,713	6.0[i]	−11.2
HLA-A2	HTLV-1 Tax	11–19	LLFGYPVYV	TAX-9	1HHK[h]	−1,774	11.0[i]	−10.9
HLA-A2	HIV-1 reverse transcriptase	309–317	ILKEPVHGV	RT-9	1HHJ[h]	−1,769	242.0[i]	−9.0
HLA-A2	HIV-1 gp120	197–205	TLTSCNTSV	GP-9	1HHG[h]	−1,580	294.0[i]	−8.9

[a]Coordinate file of protein–peptide complex from the Brookhaven (Bernstein et al., 1977) Protein Data Bank (PDB).
[b]Buried solvent-accessible surface area on protein and peptide upon complexation as calculated using the program SURFCV with the PARSE van der Waals atomic radii (see Methods for details).
[c]Measured equilibrium dissociation constant of protein–peptide complex.
[d]Experimental binding free energy of protein–peptide complex calculated as $\Delta G_{exp} = RT \ln K_D$ (T = 25 °C).
[e]Fremont et al. (1992).
[f]Matsumura et al. (1992).
[g]Fremont et al. (1995).
[h]Madden et al. (1993).
[i]Altuvia et al. (1995).
[j]Ruppert et al. (1993).

Table 2. *Experimental (ΔG_{exp}) and calculated binding free energies (ΔG_{calc}) for the 8 studied MHC class I protein–peptide complexes, and detail of the values calculated for the components of ΔG_{calc}, in kcal/mol*

Complex	ΔG_{exp}	ΔG_{calc}	ΔG_{coul}	ΔG_{solv}	ΔG_{el}[a]	ΔG_{np}	$-T\Delta S_{mc}$	$-T\Delta S_{sc}$
H-2K^b/SEV-9	−11.7	−22.0	−150.9	197.7	46.8	−91.7	18.0	4.9
H-2K^b/VSV-8	−11.5 (0.2)[b]	−26.4 (−4.4)	−214.6 (−63.7)	265.2 (67.5)	50.6 (3.8)	−102.4 (−10.7)	16.0 (−2.0)	9.4 (4.5)
H-2K^b/OVA-8	−11.4 (0.3)	−26.1 (−4.1)	−154.9 (−4.0)	201.7 (4.0)	46.8 (0.0)	−101.1 (−9.4)	16.0 (−2.0)	12.2 (7.3)
HLA-A2/HBV-10	−11.6	−40.6	−161.7	198.6	36.9	−107.0	20.0	9.5
HLA-A2/FLU-9	−11.2 (0.4)	−39.3 (1.3)	−164.4 (−2.7)	196.6 (−2.0)	32.2 (−4.7)	−96.6 (10.4)	18.0 (−2.0)	7.1 (−2.4)
HLA-A2/TAX-9	−10.9 (0.7)	−41.3 (−0.7)	−160.7 (1.0)	190.0 (−8.6)	29.3 (−7.6)	−95.4 (11.6)	18.0 (−2.0)	6.8 (−2.7)
HLA-A2/RT-9	−9.0 (2.6)	−44.5 (−3.9)	−211.3 (−49.6)	232.4 (33.8)	21.1 (−15.8)	−95.1 (11.9)	18.0 (−2.0)	11.5 (2.0)
HLA-A2/GP-9	−8.9 (2.7)	−40.0 (0.6)	−190.6 (−28.9)	202.8 (4.2)	12.2 (−24.7)	−81.4 (25.6)	18.0 (−2.0)	11.2 (1.7)

[a] ΔG_{el} is the sum of ΔG_{coul} and ΔG_{solv}.
[b] The relative binding free energies ($\Delta\Delta G$) with respect to the best experimental binders (SEV-9 for H-2K^b, and HBV-10 for HLA-A2) are given in parentheses.

the structure minimized with harmonic restraints (Table 2). Oscillations in this range were also observed when ΔG_{calc} was computed using 10 structures obtained from a 10-ps molecular dynamics simulation of the H-2K^b/VSV-8 complex in vacuum at 300 K, where heavy atom positions were harmonically restrained to their original crystal positions. However, it was found that the average value of ΔG_{calc} calculated from the molecular dynamics simulation was quite stable, and that it was comparable to the value of ΔG_{calc} computed from a single structure minimized with harmonic restraints (A. Windemuth & N. Froloff, unpubl. results).

Binding free energies

Table 2 summarizes the calculated binding free energies for the 8 MHC class I protein–peptide complexes. The calculated values are significantly more negative than the experimental ones (which are all about −10 kcal/mol; Table 1) due in part to the fact that the entropic cost of fixing translational and rotational degrees of freedom has not been taken into account. This issue will be discussed further below. Comparing different complexes, it is clear from Table 2 that the variation is in the individual contributions to the binding free energy are much greater than the variation in the calculated binding free energies. The latter vary over a range of 4.4 kcal/mol for the three H-2K^b/peptide complexes, and 5.2 kcal/mol for the five HLA-A2/peptide complexes. The experimental binding free energies fall in a range of less than 0.3 kcal/mol and 2.7 kcal/mol for the H-2K^b/peptide and HLA-A2/peptide complexes, respectively (Table 1).

The calculations are reasonably successful in reproducing the small observed experimental range despite the fact that the individual free energy contributions are so large. Of these, the solvation free energy terms for the individual proteins and complexes (G_{solv}) are on the order of thousands of kcal/mol. For example, for H-2K^b/VSV-8, the solvation energy is −2583 kcal/mol for the protein, −180 kcal/mol for the peptide and −2498 kcal/mol for the complex. These yield a value of ΔG_{solv} for H-2K^b/VSV-8 of 265 kcal/mol, which should be compared to the values of ΔG_{solv} near 200 kcal/mol for the other H-2K^b complexes. The range of values for ΔG_{coul} is also about 65 kcal/mol, leading to values of ΔG_{el} which are quite quite close for all three complexes (within approximately 5 kcal/mol). The individual values for ΔG_{np} are approximately −100 kcal/mol but they fall within a range 10 kcal/mol. Given the size of the individual terms, it is quite remarkable that the values of ΔG_{calc} are so close to one another. Moreover, the same is true for the HLA-A2 complexes where a comparable range of calculated binding free energies is obtained.

Despite the success in obtaining a small range for the binding free energies, the correct order of peptide binding is not reproduced for either protein. This is not surprising given the uncertainties in the calculations. The calculations also do not succeed in reproducing the fact that both H-2K^b and HLA-A2 have very similar binding free energies with their respective peptides. As will be discussed below, this may be due to differences in the details of the crystal structure determination for each set of complexes and to different reorganization free energies upon complexation.

A major conclusion of our study is that electrostatic interactions oppose binding, a result that was obtained for all the complexes that were studied (Table 2). For example, H-2K^b has a net charge of about −8 at pH = 7, VSV-8 has a charge of +1, SEV-9 has a charge of 0 (no ionizable side-chains) and OVA-8 has a net charge of 0 (two oppositely charged side-chains). These result in very different Coulomb energies for the complexes: −214.6 kcal/mol for VSV-8, −150.9 kcal/mol for SEV-9 and −154.9 kcal/mol for OVA-8 (Table 2). That the three peptides show quite similar electrostatic association energies in water (46.8 kcal/mol for both SEV-9 and OVA-8, 50.6 kcal/mol for VSV-8; Table 2) is due to a compensation between the Coulomb and solvation free energies. The added Coulomb attraction for VSV-8 is compensated by a loss in solvation free energy for this charged peptide. Both hydrogen bonding and ionizable groups contribute to the electrostatic term with the former making the dominant contribution. Thus, the unfavorable electrostatic contributions to binding are due primarily to the desolvation of hydrogen bonding groups which is not fully compensated by hydrogen bond formation in the complex.

The loss of main-chain and side-chain configurational entropy strongly opposes binding. The main-chain term, $-T\Delta S_{mc}$, is quite large for all complexes (about half of the electrostatic term) and is proportional to the number of residues in the peptide. The side-chain term, $-T\Delta S_{sc}$, is surprisingly small even though numerous side chains are in the peptide-protein interface. (For example, 96% of the buried H-2K^b atoms are from side chains, and 23 different side chains of H-2K^b are in van der Waals contact with VSV-8; Fremont et al., 1992). However, most of the side chains in the binding site of both H-2K^b and HLA-A2 are fairly well shielded from solvent even prior to association so that according to the criteria of Pickett & Sternberg (1993) (see Methods) they are fairly

immobile even in the isolated protein. Most of the entropic loss is from the peptide side chains which are all solvent-exposed and, with the exception of one to three side chains per peptide, are buried upon binding. Indeed, about 80% of the peptides' solvent-accessible surface becomes buried upon MHC binding (Stanfield & Wilson, 1995). Differences in configurational entropies of individual side chains lead to different values for the $-T\Delta S_{sc}$ term (Table 2) but these variations are small (<8 kcal/mol) relative to the variations of the other binding free energy terms.

Protein–peptide association appears to be driven by the hydrophobic effect (ΔG_{np}), which is large enough to compensate for the unfavorable electrostatic contribution as well as for the loss of side-chain and main-chain configurational entropy. Interestingly, the nonapeptide SEV-9 buries less solvent-accessible surface area upon binding than the two octapeptides ($\Delta(CA)$, Equation 3, is equal to 1576 Å^2 for SEV-9, 1759 Å^2 for VSV-8, and 1737 Å^2 for OVA-8). SEV-9 bulges out of the binding groove so that its extra residue can be accommodated by H-2K^b (Fremont et al., 1992). As a result, SEV-9 is the only peptide among the three for which two side chains retain more than 60% accessibility to water upon binding (Pro^{P3} and Asn^{P5}). In contrast, for the five HLA-A2/peptide complexes, the longest peptide (HBV-10) buries the most surface area and hence has the largest value for ΔG_{np}. For these complexes increased nonpolar contributions are opposed by increased electrostatic contributions favoring dissociation, resulting in a fairly small range of calculated binding free energies.

Discussion

In this paper we have employed a physical model that involves a static but atomic level description of interacting molecules, a continuum solvent model, a simple estimate of configurational entropies, and a parameter set which was derived from independent observations. Conformational changes upon binding are included in a strain free energy term, ΔG_{strain}, which is never calculated explicitly. Configurational entropy losses upon binding are treated with a simple rotamer model but more complex dynamical contributions to the entropy before and after complexation are ignored. The approach has been put to a rather severe test, the calculation of the relative binding free energies of different peptides to two MHC class I proteins. Although the approximations inherent in this treatment as well as uncertainties in the energetic parameters render it unlikely that the calculated results will be in close agreement with experiment, this is not a goal of the present study. Rather, we have attempted to explore the consequences of applying a complete physical model to the calculation of binding free energies with the goal of determining both the strengths and limitations of the model.

We have chosen to study MHC protein–peptide complexes, in part due to their inherent interest and in part due to the challenge posed by calculating the binding free energies of rather different peptides. We first consider the absolute binding free energies that are calculated and then consider the relative binding free energies of different complexes. With regard to absolute binding free energies, the a priori assumption is that the calculated values will be too negative since we have ignored the positive contributions from ΔG_{strain} and from the free energy term which accounts for the loss of translational and rotational degrees of freedom upon binding, $\Delta G_{t,r}$. The value of $\Delta G_{t,r}$ depends on the assumption made as to the tightness of binding, as has been discussed (Finkelstein & Janin, 1989; Gilson et al., 1997). $\Delta G_{t,r}$ is usually considered to be a constant for ligands that bind to the same protein, and estimates for its magnitude typically range between 7 and 15 kcal/mol for protein-sized systems (Steinberg & Scheraga, 1963; Page & Jencks, 1971; Erickson, 1989; Finkelstein & Janin, 1989; Novotny et al., 1989; Searle et al., 1992; Tidor & Karplus, 1994; Janin, 1995). Adding ~10 kcal/mol to ΔG_{calc} yields a binding free energy that is more negative than the experimental value by about 5 kcal/mol for the H-2K^b complexes and about 20 kcal/mol for the HLA-A2 complexes. The remaining difference is presumably due to ΔG_{strain}, and to a variety of errors in the theoretical procedure, crystallographic coordinates, energetic parameters and physical model.

ΔG_{strain} accounts for the unfavorable conformational free energy that the protein and ligand undergo in order to achieve optimal binding. While this term must always be present, its magnitude is difficult to estimate. In fact, no peptide-free MHC class I protein crystal structure has been reported to date, so that only indirect evidence is available as to the protein structural changes that accompany peptide binding (Elliott et al., 1991; Bluestone et al., 1992, 1993; Catipovic et al., 1992, 1994; Sherman et al., 1993; Rohren et al., 1994). However, they are likely to be similar for the same protein interacting with different peptides, which is consistent with the fact that the protein conformation of both H-2K^b and HLA-A2 shows only minor conformational changes in comparing one protein–peptide complex to another (Fremont et al., 1992, 1995; Madden et al., 1993).

The difference in ΔG_{calc} for the H-2K^b and HLA-A2 complexes may be due to a number of sources including differences in the model building and refinement procedures used to obtain the crystal coordinates of the complexes. Fremont et al. (1992, 1995) used the crystallographic R values to refine the structures of the H-2K^b complexes, while Madden et al. (1993) preferred to minimize the R_{free} (Brünger, 1992) values of the HLA-A2 complexes. Furthermore, Fremont et al. explicitly included observed crystallographic waters in the models (about 100 per MHC protein–peptide structure) while Madden et al. did not. As a test, we performed a restrained energy-minimization of the H-2K^b/peptide complexes without crystal waters and obtained ΔG_{calc} values about 8 kcal/mol lower than the ones calculated from the structures minimized with water molecules (results not shown). Thus, this factor alone may account for much of the difference in the calculated values for the two sets of complexes.

An upper limit to ΔG_{strain} should correspond to the folding free energy of most proteins of about 10 kcal/mol, because the free energy difference between folded, partially folded, and unfolded forms of any protein must be in this range. Assuming half this value for ΔG_{strain} and adding 10 kcal/mol for $\Delta G_{t,r}$ implies that about 15 kcal/mol should be added to the calculated binding free energies in Table 2. When this is done, the absolute values for the H-2K^b complexes appear to be in good agreement with experiments while those for the HLA-A2 complexes are still somewhat too negative. Given the size of the individual contributions to the binding free energy, the fact that the calculated values are of a reasonable magnitude is reassuring.

It is also encouraging that the calculated binding free energies for a single set of complexes are so similar. Despite the neglect of ΔG_{strain} and precision problems arising from subtracting large free energy contributions, the calculated binding free energies for different peptides binding to the same protein are extremely close to one another. This is a rather striking result that, taken together with the apparently reasonable values for ΔG_{calc} that we have obtained, suggests that the underlying physical model we have used and the

assumptions we have made have some validity. Moreover, the calculated binding free energies are all more negative than the experimental values, which is consistent with the neglect of positive free energy contributions to the association free energy. It is important to emphasize in this regard that all parameters used in the calculations were derived from a totally independent set of observations (e.g., PARSE parameters are based on the solvation free energies of small organic molecules; Sitkoff et al., 1994).

The success of the approach used in this work, as measured by the similar values obtained for the different peptides and by the fact that the values themselves are in the expected range, has a number of important implications for future work. First, that the model itself is reasonable implies that it can be used to elucidate essential features of the binding process. Second, it suggests that the approach might be successful in the treatment of simpler problems, for example in predicting the relative binding free energies of chemically similar and less flexible substrates, as has been recently shown for a few protein–ligand systems (Wendoloski et al., 1993; Shen & Quiocho, 1995; Shen & Wendoloski, 1995; Zhang & Koshland, 1996). Third, it can be used to validate simpler approximations based on a similar physical model, for example the one used by Vajda et al. (1994) in their study of MHC–peptide interactions.

It is of interest to consider the determinants of MHC–peptide binding based on the results of this paper. As has been found in previous work based on the application of continuum solvent models, electrostatic interactions, including hydrogen bonding interactions, are found to oppose binding (see, e.g., Yang & Honig, 1995a, 1995b, for a discussion of similar effects in the context of protein folding). This result follows from the description of binding and folding processes in terms of the removal of polar and nonpolar groups from water. It is generally found that the desolvation of individual polar groups is rarely compensated by the formation of hydrogen bonds in the interior of a protein or a protein–ligand complex. It has been argued that this result is at odds with experiments based on site-directed-mutagenesis in which one member of a hydrogen bonded pair is removed (Myers & Pace, 1996). However, as has been discussed in detail, experiments of this type cannot measure the strength of hydrogen bonds directly and the results are in fact consistent with theoretical analysis (Honig & Yang, 1995).

Nonpolar interactions are found in this work to provide the major driving force for complexation, compensating both for the unfavorable electrostatic interactions and for the various entropy losses associated with the binding process. These nonpolar interactions result from the hydrophobic effect and from the possible effects of close packing in the complex which are likely to be stronger than solute–solvent van der Waals interactions for the unbound species (see the discussion by Friedman & Honig, 1995). The calculated values of ΔG_{np} of about -95 kcal/mol result from the burial of both polar and nonpolar surface area, totaling approximately 1650 Å^2 of curvature-corrected solvent-accessible surface area per complex. This is partitioned into approximately two-thirds buried nonpolar area (i.e., about -60 kcal/mol) and one-third buried polar area (i.e., about -35 kcal/mol each). If we add the nonpolar term due to polar atoms to ΔG_{el} (which is about 35 kcal/mol on average; Table 2), we find that the net free energy contribution of polar atoms to binding is close to zero. Thus, it is the burial of approximately 1050 Å^2 nonpolar surface area that actually drives the binding process. Almost all of the nonpolar contribution is due to the burial of side-chain atoms. In a future publication we will consider the contributions of individual residues in detail with the goal of elucidating the principles of MHC protein–peptide recognition.

Methods

Theory

The binding free energy can be written in the form:

$$\Delta G_b = \Delta G_{el} + \Delta G_{np} + \Delta G_{strain} - T\Delta S_{mc} - T\Delta S_{sc} - T\Delta S_{t,r} \quad (1)$$

ΔG_{el} and ΔG_{np} are the electrostatic and nonpolar contributions to binding obtained from the thermodynamic cycle in Figure 1. ΔG_{strain} accounts for possible distortions in the protein or peptide upon complexation. $T\Delta S_{mc}$ and $T\Delta S_{sc}$ describe the loss of configurational entropy due to the loss of backbone and side-chain torsional freedom upon complexation. $T\Delta S_{t,r}$ accounts for the loss of translational and rotational degrees of freedom upon binding.

The method used to calculate the electrostatic free energy, ΔG_{el}, has been described previously (Gilson & Honig, 1988; Smith & Honig, 1994) and is summarized in Figure 2. ΔG_{el} is obtained from the sum of the Coulomb and reaction field (solvation) energies of the complex minus that of the isolated reactants:

$$\Delta G_{el}(\epsilon_i, \epsilon_o) = \Delta G_{coul}(\epsilon_i) + \Delta G_{solv}(\epsilon_i, \epsilon_o) \quad (2)$$

where $\Delta G_{el}(\epsilon_i, \epsilon_o)$ is the total change in electrostatic free energy for the two reactants with interior dielectric constant ϵ_i, both of which are embedded in a solvent of dielectric ϵ_o. $\Delta G_{coul}(\epsilon_i)$ is the pairwise Coulomb free energy between the two interacting molecules which are embedded in a medium of dielectric constant ϵ_i. $\Delta G_{solv}(\epsilon_i,\epsilon_o)$ is the change in the reaction field free energy upon transferring the various species from a medium of dielectric ϵ_i to one of dielectric ϵ_o.

The nonpolar (hydrophobic) contribution to the binding free energy (Fig. 1) is given by

$$\Delta G_{np} = \gamma_{aw} \Delta(CA) \quad (3)$$

where γ_{aw} is the microscopic surface tension associated with the transfer of alkane from liquid alkane to water. The curvature correction factor, C, describes the effect of the curvature of the molecule on its interfacial free energy (Nicholls et al., 1991; Sharp et al., 1991) while A is the solvent-accessible surface area (Lee & Richards, 1971). C for an entire molecule is obtained as an average over local curvature factors, c, which in turn are related to the solid angle that is excluded to a sphere with the radius of a water molecule when in contact with the molecular surface (Nicholls et al., 1991). For a spherical surface patch of radius R, c is given by

$$c = 1/(1 \pm a/R) \quad (4)$$

where a is the radius of the water probe, the positive sign applies to a convex region and the negative sign to a concave region. The concave regions of a solvent-accessible surface are more hydrophobic than the convex regions since water molecules are more restricted in the former case. Van der Waals interactions are not considered explicitly; any difference in protein–ligand and solute–solvent van der Waals interactions is assumed to be implicitly taken into account in the surface tension parameter, γ_{aw}.

$-T\Delta S_{sc}$ is calculated using the empirical scale of Pickett & Sternberg (1993). Their model assumes that a solvent-exposed

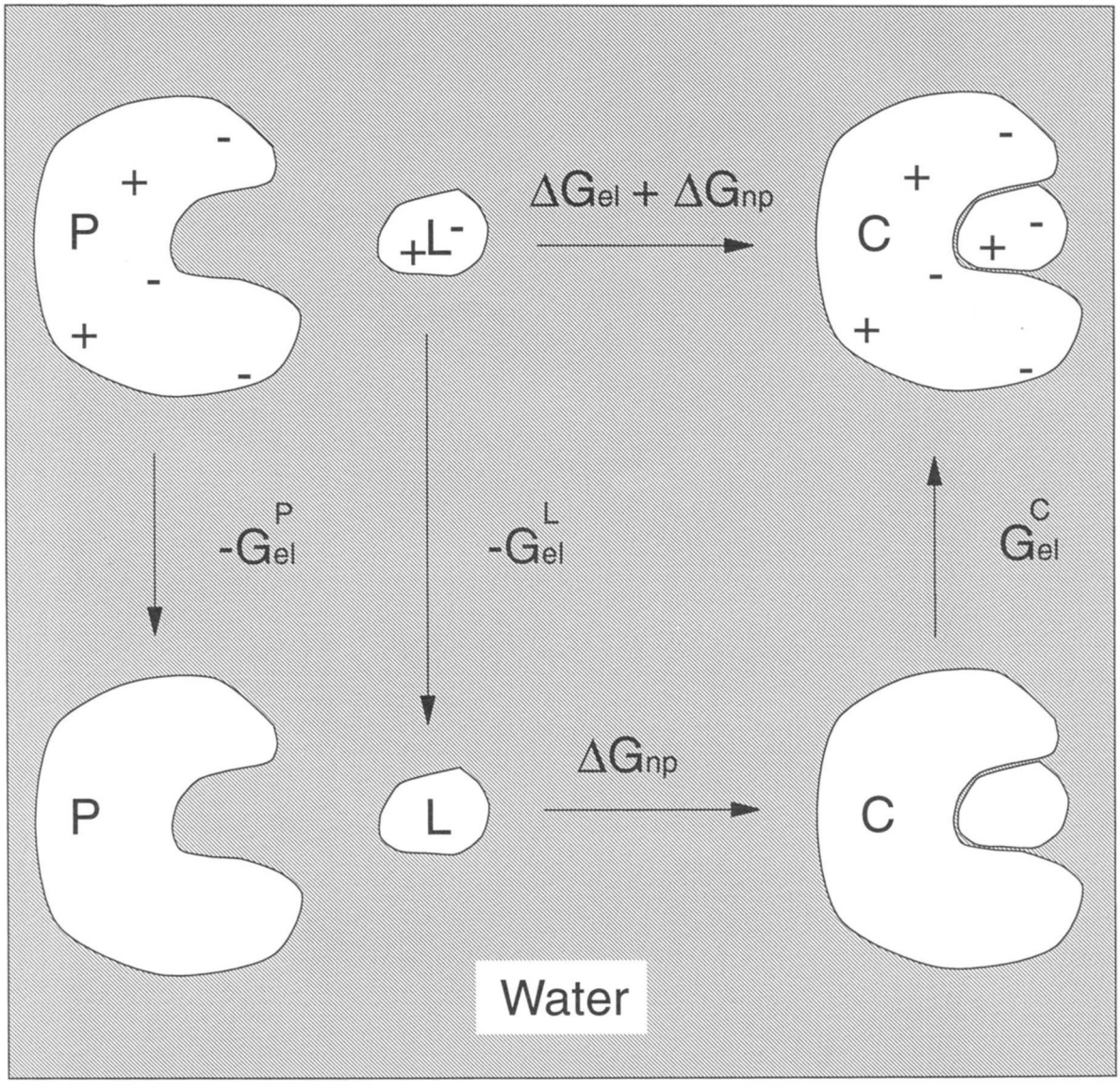


Fig. 1. Thermodynamic cycle for calculation of the electrostatic (el) and nonpolar (np) contributions to the binding free energy (ΔG) of a protein (P) and a ligand (L) to form a macromolecular complex (C) in aqueous solution.

side-chain (whose relative accessibility, *RA*, is greater than 60%) rotates freely, whereas a buried side chain ($RA < 60\%$) is restrained to one rotamer. *RA* is defined as

$$RA = \frac{\text{side-chain accessible area}}{\text{side-chain area in fully extended state}}. \quad (5)$$

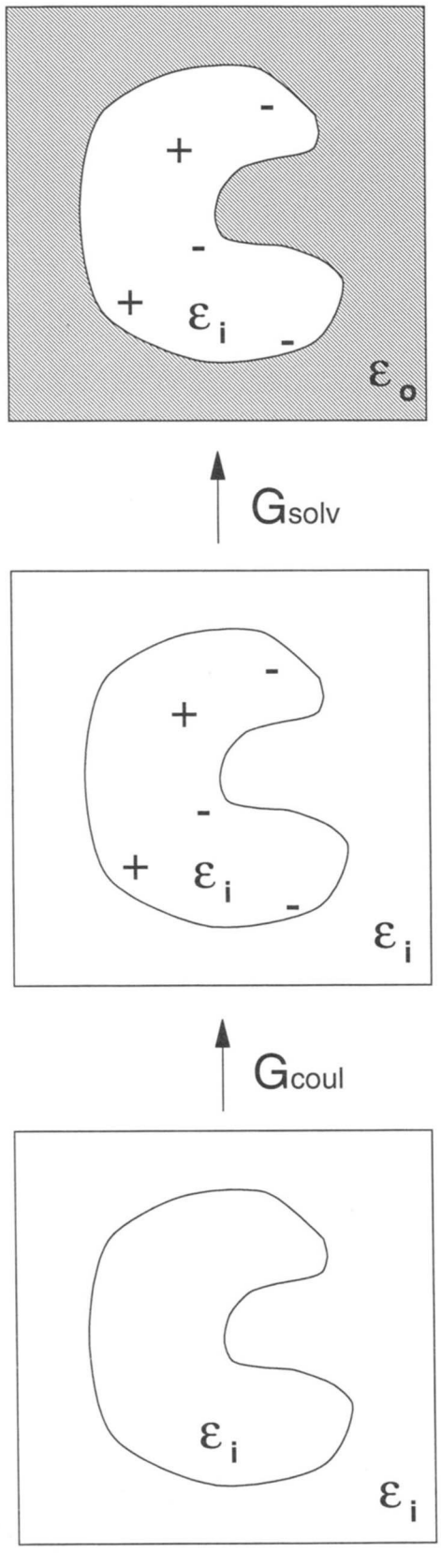
The empirical scale of Pickett and Sternberg (1993) gives the configurational entropies of the 20 amino acid side chains obtained from the observed distributions of side-chain rotamers in 50 non-homologous protein crystal structures. This scale correlates well with empirical scales obtained by other authors using different techniques (Doig & Sternberg, 1995). It predicts an entropic cost of 0.54 kcal/mol per CH_2 for fixing a carbon–carbon single bond to a single rotamer. This value is within the range expected if one assumes that each single bond occupies between two and three conformational minima when rotating freely ($R \ln 2$ and $R \ln 3$ are 0.42 and 0.66 kcal/mol at 300 K, respectively). It is also close to the estimated entropic cost of 0.45 kcal/mol/CH_2 at 25 °C of fixing side chains upon the liquid to solid phase transition of alkanes (Nicholls et al., 1991). Configurational entropy changes are calculated for the side chains of both the MHC proteins and their peptidic ligands by determining which solvent-exposed side chains become buried upon binding.

$-T\Delta S_{mc}$ is assumed to be 2 kcal/mol/residue. This value was determined from a recent theoretical study of α-helix formation (Yang & Honig, 1995a) and is in the range predicted by other estimates (Honig & Yang, 1995, and references therein).

We define ΔG_{calc}, which is the calculated binding free energy, as

$$\Delta G_{calc} = \Delta G_{el} + \Delta G_{np} - T\Delta S_{mc} - T\Delta S_{sc} \quad (6)$$

ΔG_{calc} will be smaller than ΔG_b (Equation 1) because the two terms that have been ignored in the former, ΔG_{strain} and $-T\Delta S_{t,r}$



are both positive. Thus, ΔG_{calc} is expected, a priori, to be more negative than the experimental binding free energy.

Calculations and parameters

The DelPhi program with a new molecular surface generation algorithm (Sridharan et al., 1997) was used for the finite difference Poisson-Boltzmann (FDPB) calculations (Nicholls & Honig, 1991). The precision of the FDPB calculations is strongly dependent on the method used to generate the molecular surface and it is extremely important to use an accurate surface generation algorithm in binding energy calculations. Atomic charges and radii were taken from the PARSE parameter set (Sitkoff et al., 1994), which accurately reproduces the vacuum-to-water transfer free energies of amino acid side-chain and peptide backbone analogs, assuming $\epsilon_i = 2$ and $\epsilon_o = 80$. An interior dielectric constant of 2 was assigned to both the peptides and proteins.

Dielectric constants of 4 are frequently used to account for the dielectric response due to conformational changes that are not treated explicitly (see, e.g., Sharp & Honig, 1990) but we have preferred here to calculate the binding free energy for rigid body association and assume that all conformational changes upon binding are incorporated in the ΔG_{strain} term. Under these circumstances, a dielectric constant of 2 is appropriate since this value accounts for electronic polarizability but not for structural reorganization. In addition, the use of a dielectric of 4 is intended as a means of accounting for the screening of pairwise Coulomb interactions due to small conformational changes while in the calculation of binding free energies one is primarily concerned with the conformational relaxation of the separate monomers following dissociation of the complex (which may be viewed as reducing the "self energy" of the monomers). Using a dielectric constant of 4 in this case does not account for this relaxation (indeed, the calculated effect of the higher dielectric is to strengthen binding while relaxation of the monomers weakens binding).

DelPhi was run on a cubic grid with 155 to 165 grid points per side such that a resolution of 2 grid points per Å and a percentage grid fill of 90% was maintained in all calculations. Dummy atoms were used to ensure that a given complex and the corresponding protein and peptide had an identical scale and position on the grid. Solutes were treated as polarizable cavities of shapes defined by their molecular surfaces (a probe radius of 1.4 Å for water was used). Observed crystallographic waters whose coordinates are specified in the H-2K^b models (about 100 per complex), but not in the HLA-A2 models, were treated as corresponding to bulk solvent. The ionic strength was set to zero; use of the physiological value (e.g., about 0.1 M) was found to have little effect ($<$0.5 kcal/mol) on the magnitude of ΔG_{calc}.

Plain and curvature-corrected solvent-accessible surface areas (respectively used to calculate $-T\Delta S_{sc}$ and ΔG_{np}) were computed from atom radii with a probe radius set at 1.4 Å using the numerical algorithm implemented in the program SURFCV (Sridharan

Fig. 2. Thermodynamic process for the calculation of the total electrostatic free energy of a molecule (Gilson & Honig, 1988). G_{coul} is the free energy of assembling the atomic charges from infinity in a medium of dielectric constant equal to that of the macromolecule's interior (ϵ_i). G_{solv} corresponds to the free energy of bringing the solvent boundary from infinity to the position defined by the molecular surface (Richards, 1977) of the molecule.

et al., 1992). Unpublished tests showed that the plain solvent-accessible surface area of a typical MHC protein–peptide complex calculated with the maximal resolution available in SURFCV and with the exact analytical surface algorithm implemented in the program PMD (Windemuth & Schulten, 1991; Board et al., 1992; Windemuth, 1995) agree to within 0.03%. We used the numerical algorithms of SURFCV since there is no available analytical algorithm to compute curvature-corrected solvent-accessible surface areas. The value of γ_{aw} was set at 58.18 cal·mol^{-1}·Å^{-2}, which, when used with PARSE parameters, reproduces water-to-alkane transfer free energies and is thus consistent with the cycle shown in Figure 1. This value is close to the one estimated by Nicholls et al. (1991) (~60 cal·mol^{-1}·Å^{-2}) based on a model for the curvature-dependence of the hydrophobic effect. The actual value for γ_{aw} depends on the set of atomic radii used to calculate the solvent-accessible surface area (Friedman & Honig, 1995).

Structure selection and modeling

Binding energy calculations were carried out on three crystal structures of the murine MHC class I protein H-2K^b complexed with three distinct peptides (Fremont et al., 1992, 1995), and on five crystal structures of the human MHC class I protein HLA-A2 (HLA-A*0201) complexed with five distinct peptides (Madden et al., 1993). The coordinates of the heavy atoms of the eight complexes were taken from the Brookhaven (Bernstein et al., 1977) Protein Data Bank (PDB). Peptide sources, sequences, abbreviations and experimental binding free energies at 25 °C are listed in Table 1. Since the four PDB files of the HLA-A2/nonapeptide complexes each specified coordinates for two distinct complexes, the binding free energies were calculated on both complexes and averaged.

Hydrogen atom positions were built into each X-ray crystal structure using the routine HBUILD (Brünger & Karplus, 1988) of the X-PLOR 3.1 program (Brünger, 1995), and energy-minimized with the CHARMM 22 parameter set (Brooks et al., 1983; MacKerell et al., 1992) available in X-PLOR. The coordinates of all atoms were then energy-minimized using X-PLOR and CHARMM 22. A harmonic potential with a force constant of 3 kcal·mol^{-1}·Å^{-2} was applied to all heavy atoms to restrain their positions to the original PDB coordinates. The root mean square (RMS) difference between the original PDB coordinates and the minimized coordinates of the heavy atoms in a given complex remained within 0.3 Å. This is well within the estimated crystal coordinate errors, since individual atoms in a typical protein crystal move by 0.3–0.5 Å RMS as derived from their Debye-Waller temperature factors (Luzzati, 1952; Finkelstein & Janin, 1989). By contrast, an energy minimization without restraints produced structures that deviated significantly from the original PDB coordinates, with 1.6 Å RMS for the heavy atoms.

The restrained minimization step was carried out so as to eliminate possible steric overlap as well as severe deviations from standard polypeptide geometry, with only minor deviation from the unrefined structure. In addition, the minimization effectively standardizes all the structures, for example rendering all hydrogen-bond lengths effectively identical. Small differences in these lengths can produce significant changes in calculated energies that are unlikely to be physically meaningful. We indeed observed that the seven H-bonds that are conserved between the protein and the peptide backbone for the three H-2K^b/peptide complexes (Fremont et al., 1992, 1995) show significantly different lengths from one structure to another. For example, the acceptor-donor distance in the conserved H-bond between the main-chain CO at peptide position P1 and Tyr^{159}-OH is 2.58 Å for the OVA-8 complex, but 2.91 Å for SEV-9. After restrained minimization, these distances become closer to one another (2.93 and 2.96 Å, respectively) and more optimal in length (Ippolito et al., 1990). More generally, the acceptor-donor distances of the seven conserved H-bonds show standard deviations from one complex to another that are reduced to half their original value after minimization (about 0.04 Å, as compared to about 0.07 Å before minimization).

Note added in proof

Calculations have been repeated for files 2VAA and 2VAB now in the PDB. The results for ΔG_{calc} differed from those obtained for 1VAA and 1VAB, respectively, by about 0.6 kcal/mol. More substantial but compensatory changes were found for ΔG_{coul} and ΔG_{solv} so that ΔG_{el} was basely affected. Thus, the conclusions of the paper remain the same if the more highly refined structures now in the PDB are used in the calculations.

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