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Structural Energetics of Peptide Recognition: Angiotensin II/Antibody Binding

Kenneth P. Murphy,[1] Dong Xie,[1] K. Christopher Garcia,[2] L. Mario Amzel,[2] and Ernesto Freire[1]
[1]Department of Biology and Biocalorimetry Center, The Johns Hopkins University, Baltimore, Maryland 21218, and [2]Department of Biophysics and Biophysical Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

ABSTRACT **The ability to predict the strength of the association of peptide hormones or other ligands with their protein receptors is of fundamental importance in the fields of protein engineering and rational drug design. To form a tight complex between a flexible peptide hormone and its receptor, the large loss of configurational entropy must be overcome. Recently, the crystallographic structure of the complex between angiotensin II and the Fab fragment of a high affinity monoclonal antibody has been determined (Garcia, K.C., Ronco, P.M., Verroust, P.J., Brünger, A.T., Amzel, L.M. Three-dimensional structure of an angiotensin II–Fab complex at 3 Å: Hormone recognition by an anti-idiotypic antibody. Science 257:502–507, 1992). In this paper we present a study of the thermodynamics of the association by high sensitivity isothermal titration calorimetry. The results of the experiments indicate that at 30°C the binding is characterized by (1) a ΔH of −8.9 ± 0.7 kcal mol^{-1}, (2) a ΔC_p of −240 ± 20 cal K^{-1} mol^{-1}, and (3) the release of 1.1 ± 0.1 protons per binding site in the pH range 6.0–7.3. Using these values and the previously determined binding constant in phosphate buffer, ΔG at 30°C is estimated as −11 kcal mol^{-1} and ΔS as 6.9 cal K^{-1} mol^{-1}. The calorimetric data indicate that binding is favored both enthalpically and entropically. These results have been complemented by structural thermodynamic calculations. The calculated and experimentally determined thermodynamic quantities are in good agreement. Entropically, the loss of configurational entropy is more than compensated by the entropy gain from solvent release associated with the hydrophobic effect. Enthalpically, binding is favored by polar interactions (hydrogen bonding). Consequently, the problem of binding flexible hormones is solved in much the same way as the folding of an unstructured polypeptide chain into a globular protein.** © 1993 Wiley-Liss, Inc.

Key words: thermodynamics, calorimetry, protein–hormone interaction, drug design

INTRODUCTION

The realization of structure-based drug design requires accurate quantitation of the energetics of the interaction of a ligand with a receptor, or a substrate with an enzyme, based on a knowledge of the structure of the complex. Even in cases where X-ray or NMR coordinates are available there has been only limited success at this strategy of rational design of analogs because of the inability to correlate accurately detailed energetics with structure. For design of analogs of flexible peptide hormones, this problem is further compounded by the question of how these extremely flexible molecules, which exist as an ensemble of conformations in solution, can overcome their configurational entropy to form high-affinity complexes of a single conformation with their receptors.

Angiotensin II (AII), an octapeptide hormone of sequence Asp-Arg-Val-Tyr-Val-His-Pro-Phe, is a prototype of many small, flexible peptide hormones including bradykinins, substance P, CCK, and vasopressin. AII is the primary biologically active component of the renin–angiotensin system and plays an important role in a number of physiological functions, the most prominent being blood pressure regulation. Other small peptide hormones regulate a multitude of physiological processes including vasoconstriction, ionotropic effects, pain, hunger, and lactation, and have therefore been the targets of

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Address reprint requests to Dr. Ernesto Freire, Department of Biology and Biocalorimetry Center, The Johns Hopkins University, Homewood Campus, Charles & 34th Streets, Baltimore, MD 21218.
K. Christopher Garcia's present address is Departments of Molecular Biology and Protein Engineering, Genentech, Inc., 460 Point San Bruno Blvd., South San Francisco, CA 94080.

pharmacological efforts to design antagonists or super agonists. Hampering these design efforts has been the lack of structural information concerning the bound conformations of these molecules.

Recently the three dimensional structure of a complex of AII with the Fab fragment of a high affinity antibody revealed a bound conformation of AII which is remarkably similar to predicted receptor-bound conformations of this molecule.[1] In an attempt to characterize the energetics and mode of association of AII to the antibody molecule, we have estimated the thermodynamic parameters of binding (ΔH, ΔS, and ΔC_p) from direct calorimetric measurements. In addition, we have performed detailed structural thermodynamic calculations utilizing the crystallographic coordinates of the complex.

MATERIALS AND METHODS

The high affinity IgG (Ab 131) was obtained courtesy of Drs. P. Verroust and P.M. Ronco in buffer (10 mM phosphate, 20 mM NaCl, pH 7) at a concentration of 6 mg ml^{-1} and stored frozen until use. Human AII was obtained from Peninsula Laboratories (Belmont, CA) and was dissolved in the appropriate buffer for the titration experiments. All buffers were prepared at a concentration of 20 mM with 50 mM NaCl and adjusted to the desired pH with HCl or NaOH solution as required. For each experiment an aliquot of IgG stock solution was diluted in the appropriate buffer and dialyzed against 4 liters of buffer overnight at 4°C. The IgG concentration was determined using A_{280} = 1.414 for a 1 mg ml^{-1} solution and a molecular mass of 150 kDa.

Calorimetric experiments were performed in an Omega titration microcalorimeter (Microcal Inc., Northhampton, MA). A Keithley 181 nanovoltmeter was used as a preamplifier to improve the signal to noise ratio of the instrument. The reaction cell (cell volume 1.4 ml) was filled with a 5.7 μM solution of IgG and a series of 10 μl (4.2 nmol) injections of AII solution were made. Injections were continued until no further heat effect was observed upon subsequent titration. Analysis of the data was performed using software developed in this laboratory as described earlier.[2] The heat capacity change upon binding, ΔC_p, was determined from the temperature dependence of the binding enthalpy. Protonation effects were investigated by experiments in buffers with different enthalpies of ionization as listed in Table I.

Circular dichroism spectra were recorded in a Jasco J-710 instrument (Japan Spectroscopic Co., Ltd., Tokyo, Japan) using a 0.5 mm cell. The experiments were performed at 25°C with a protein concentration of 0.27 mg/ml in phosphate buffer at pH 7.3. A solution of IgG saturated with AII was prepared by adding 1 μl of a 1.8 mM AII solution to a 1-ml aliquot of the IgG solution. Spectra were analyzed using Jasco software.

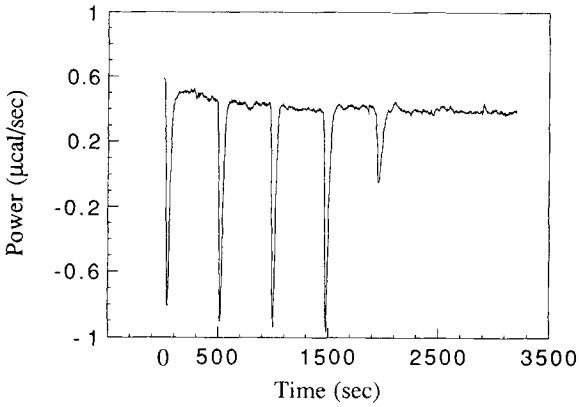

Fig. 1. High-sensivity isothermal calorimetric titration of IgG with angiotensin II. The power output, in units of μcal s^{-1}, is shown as a function of time for successive injections of angiotensin II. For each injection the heat evolved is given from the area under the peak. The injection volume was 10 μl (4.2 nmol) into a 5.7 μM solution of IgG in buffer (20 mM MOPS, 50 mM NaCl, pH 7.3). The cell volume was 1.4 ml.

RESULTS AND DISCUSSION

Calorimetry

A calorimetric titration of Ab 131 with AII is shown in Figure 1. This titration is characterized by a significant exothermic heat effect with each injection until the binding sites become saturated with ligand. After this point, subsequent injections show no significant heat effect. The binding enthalpy, $\Delta H_{binding}$, is equal to the total heat evolved in saturating all the sites divided by the total number of binding sites in the reaction cell (two sites per IgG). Under the conditions of these experiments the concentrations required for calorimetric measurements are higher than the dissociation constant of the AII–antibody complex, thus precluding a direct calorimetric determination of the binding affinity. This situation was confirmed by comparing the enthalpy change obtained from the heat released by a single injection divided by the moles of ligand injected, and the binding enthalpy determined from the total heat (sum of all the peaks) divided by the number of binding sites in the reaction cell. Equivalence of these terms indicates that all the ligand added to the reaction cell is bound upon each injection. For the experiment presented in Figure 1 we calculate a $\Delta H_{binding}$ of -16.2 ± 0.8 kcal mol^{-1} whereas the binding enthalpy estimated from the first peak is -15.8 ± 0.8. The similarity between these two quantities indicates that the titration is performed under conditions of total association at partial saturation and that the association constant cannot be determined from the analysis of the calorimetric titration data.[3] Since $\Delta H_{binding}$ was not determined by fitting the titration curve to a binding model, the primary source of error in the determination of $\Delta H_{binding}$ is the error in the protein concentration which is estimated at approximately 5%.

TABLE I. Binding Heats for the Interaction of Angiotensin II With Fab*

Temperature (°C)	pH	Buffer	ΔH_{ion} (kcal mol^{-1}) (30°C)	$\Delta H_{binding}$ (kcal mol^{-1})
30.5	6.0	Cacodylate	−0.56	−7.6 ± 0.4
30.5	6.0	MES	3.73	−13.6 ± 0.7
30.5	6.0	ACES	7.47	−15.8 ± 0.8
30.5	7.3	Phosphate	1.00	−9.7 ± 0.5
30.5	7.3	PIPES	2.76	−12.7 ± 0.6
30.5	7.3	MOPS	5.32	−16.2 ± 0.8
30.5	7.3	Tricine	7.66	−17.6 ± 0.9
19.4	7.3	Tricine	7.66	−15.2 ± 0.8
9.8	7.3	Tricine	7.66	−12.7 ± 0.6

*The buffer concentration is 20 mM with 50 mM NaCl.

There are two quantities that need to be evaluated for an experimental dissection of the enthalpy change. First is the existence of a ΔC_p for the reaction and second is whether the reaction is linked to a protonation/deprotonation event. The value of ΔC_p is obtained by measuring $\Delta H_{binding}$ at different temperatures and the effects of protonation/deprotonation are determined by performing measurements in buffers with different enthalpies of ionization. The results of these experiments are summarized in Table I.

The data from Table I show that $\Delta H_{binding}$ varies linearly with temperature. A plot of $\Delta H_{binding}$ versus temperature for the IgG/AII reaction (Fig. 2) reveals that the heat capacity change, ΔC_p, given by the slope of the line, is -240 ± 20 cal K^{-1} mol^{-1}.

The data from Table I also indicate that at a single temperature the binding enthalpy is a function of the enthalpy of ionization of the buffer. If the binding reaction involves a change in protonation, the measured $\Delta H_{binding}$ is the sum of the reaction binding enthalpy, ΔH_{react}, which is independent of the buffer, and a term proportional to the enthalpy change of ionization of the buffer,

$$\Delta H_{binding} = \Delta H_{react} + N_{H^+} \Delta H_{ion} \quad (1)$$

where N_{H^+} is the number of protons released by the buffer and ΔH_{ion} is the ionization heat of the buffer. Thus, experiments performed in various buffers at constant pH can be used to construct a plot of $\Delta H_{binding}$ versus ΔH_{ion} (Fig. 3) in which the slope yields the number of protons released by the buffer (N_{H^+}) and the intercept yields ΔH_{react}. Analysis of the data indicates that approximately one proton (1.1 ± 0.1) is released to the buffer per binding site in the pH region studied; the intercept, ΔH_{react}, is -8.9 ± 0.7 kcal mol^{-1}.

The binding constant for the AII interaction with the Fab fragment of the IgG has previously been determined at 4°C in phosphate buffer[4] as 7.4×10^9 M^{-1}, corresponding to a ΔG of -12.5 kcal mol^{-1} Using the experimental values of ΔC_p and ΔH in phosphate buffer from Table I, ΔG adjusted to 30°C

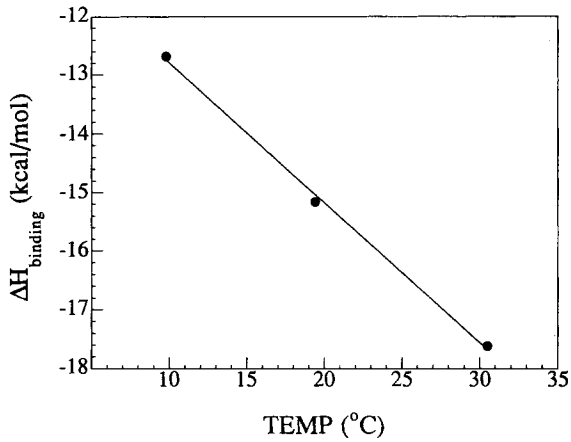

Fig. 2. Enthalpy change of binding, $\Delta H_{binding}$, of angiotensin II to IgG as a function of temperature. The data are given in Table I. The solid line is the linear least-squares fit of the data. The heat capacity change, given by the slope, is -240 ± 20 cal K^{-1} mol^{-1}.

is calculated to be -11.8 kcal mol^{-1}, or -11.0 kcal mol^{-1} after correcting for ΔH_{ion} of phosphate. This results in a ΔS value of 6.9 cal K^{-1} mol^{-1}.

Structural Energetic Calculations

Heat capacity and enthalpy changes

Recently, it has been shown that the major polar and apolar contributions to the enthalpy[5,6] and heat capacity changes[5–9] for protein folding/unfolding transitions can be parametrized in terms of the change in accessible polar and apolar surface area associated with the folding/unfolding process. The effects of protonation and other specific effects are considered separately[5,6,10]. Since the association between AII and the antibody molecule involves the same basic interactions found in protein folding, we decided to test whether the same set of parameters can also account for the ΔH and ΔC_p of binding.

In this approach, the most fundamental parameter is ΔC_p which is directly proportional to the change in polar and apolar surfaces accessible to the

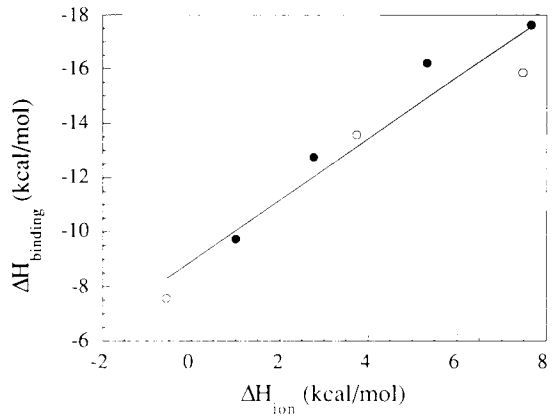

Fig. 3. Enthalpy change of binding, $\Delta H_{binding}$, of angiotensin II to IgG as a function of the ionization enthalpy, ΔH_{ion}, of the buffer from data given in Table I. Ionization enthalpies were taken from H. Fukada and K. Takahashi (personal communication) and from Christensen et al.[13] The open circles are data at pH 6 and the filled circles are data at pH 7.3. The solid line represents linear least-squares fitting of these points. The binding enthalpy in a buffer with zero ionization enthalpy, given by the intercept is −8.9 kcal mol^{-1}. The number of protons released by buffer upon binding, given by the slope, is −1.1 protons per binding site.

solvent.[5–9] The heat capacity change, ΔC_p, is given by[5,6]

$$\Delta C_p = \Delta C_{p,\,ap} + \Delta C_{p,\,pol} \quad (2a)$$

$$= (0.45 \pm 0.02)\,\Delta A_{ap} - (0.26 \pm 0.03)\,\Delta A_{pol} \quad (2b)$$

where $\Delta C_{p,\,ap}$ and $\Delta C_{p,\,pol}$ are the apolar and polar contributions to the heat capacity change, ΔA_{ap} and ΔA_{pol} are the changes in the buried apolar and polar surface areas upon binding, and 0.45 and −0.26 are the elementary apolar and polar contributions per mole of Å^2 in units of cal K^{-1}.

The enthalpy change is calculated with reference to the temperature at which the apolar contribution is assumed to be zero, T_H*. Thus the enthalpy change is

$$\Delta H = \Delta H^* + (\Delta C_{p,\,ap} + \Delta C_{p,\,pol})\,(T - T_H^*) \quad (3)$$

where ΔH* is the polar contribution to ΔH at T_H*. The value for ΔH* is directly proportional to the buried polar area and is given by the equation[5,6]

$$\Delta H^* = (35 \pm 3)\,\Delta A_{pol}. \quad (4)$$

The value for T_H* has been obtained from the analysis of protein folding/unfolding data as 100 ± 6°C.[7] This analysis has been shown to predict the enthalpy change for protein unfolding to within ± 6% at the median melting temperature of 60°C.[5,6]

The changes in accessible surface areas upon binding of angiotensin II to the antibody molecule were determined from the crystal structure using the Lee and Richards algorithm[11] as implemented by Dr. Scott Presnell (University of California, San Francisco), using a probe radius of 1.4 Å and a slice width of 0.25 Å. The uncomplexed state was modeled assuming an extended conformation for AII, and no significant change in the structure of the IgG. This assumption was verified by circular dichroism (CD) measurements. The far UV (190–280 nm) spectra of isolated IgG and of the IgG/AII complex are shown in Figure 4. Secondary structure analysis of the difference spectrum was compared to secondary structure content of the complexed AII (analyzed using the molecular graphics program Quanta (Polygen, Waltham, MA)) and indicates that all the changes in the CD spectra can be assigned to the structure formed in the AII. Consequently no significant structural changes are apparent in the IgG molecule upon AII binding, consistent with many other antibody complexes.[12]

The surface area calculations indicate that a total of 993 Å^2 of apolar surface area and 745 Å^2 of polar surface area are buried upon binding (ΔA_{ap} = −993 Å^2; ΔA_{pol} = −745 Å^2). Using Eq. (2b), a ΔC_p of −250 ± 30 cal K^{-1} mol^{-1} is predicted, in close agreement with the experimental value of −240 ± 20 cal K^{-1} mol^{-1}.

Using the above equations, we estimate a ΔH of −8.4 ± 0.2 kcal mol^{-1} at 30°C; the experimentally determined value at this temperature is −8.9 ± 0.7 kcal mol^{-1}. These values are close; however, the experimental value includes the ionization enthalpy of the complex, which has not been explicitly considered in the calculation.

The experimental value of −8.9 kcal mol^{-1} includes the enthalpy change of binding AII to the IgG and the heat of releasing a proton from the AII/IgG complex. The functional group which releases the proton has not been identified; however, the current results suggest that the proton is either released from a group with a pK_a of 8 or higher, or that there are two groups, each with a pK_a between 6 and 7.3, which are half occupied initially. It is feasible that, in either case, the ionization heat of the group is small,[13] so that the experimental reaction heat is close to the intrinsic heat of binding as calculated above. On the other hand, if the group has a sizable protonation enthalpy the intrinsic heat of binding would be significantly smaller.

Entropy changes

The entropy change for protein folding/unfolding transitions[5,6,14] can be written using, as the reference temperature, the temperature at which the apolar contribution to the entropy change is zero, T_S*.

$$\Delta S = \Delta S^* + (\Delta C_{p,\,ap} + \Delta C_{p,\,pol})\,\ln(T/T_S^*) \quad (5)$$

where ΔS* is the residual entropy change evaluated at T_S* and the term proportional to ΔC_p reflects solvent restructuring. The value of T_S* has been shown to be the same for all processes involving the transfer of apolar surface into water[14,15] and has a

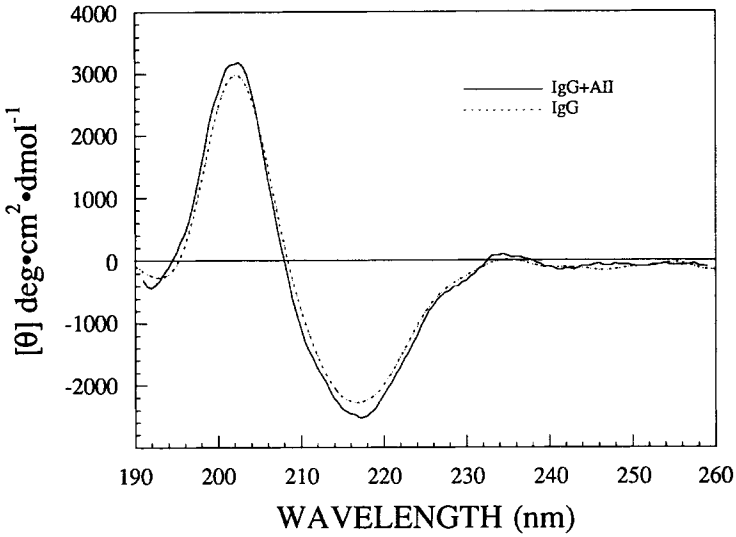

Fig. 4. Far UV CD spectra of the IgG and IgG/angiotensin II complex. The dotted curve is the CD spectrum for the unligated IgG. The solid curve is for the IgG/angiotensin II complex. Analysis of the difference spectra, normalized to the angiotensin II concentration, gives approximate secondary structure content of 17% helix, 56% sheet, 15% turn, and 11% nonregular structure. Analysis of the angiotensin II structure from the coordinates in the complex, analyzed in Quanta (Polygen, Waltham, MA), indicates comparable values of 13% helix, 38% sheet, 13% turn, and 38% nonregular structure.

value of 112 ± 1°C.[14–16] Since, by definition, there is no apolar contribution to the entropy at the reference temperature, ΔS^* reflects configurational and other statistical contributions to ΔS. For the binding of a flexible peptide to a protein we need to consider primarily: (1) the change in backbone and side chain conformational degrees of freedom of the peptide (ΔS_{pep}), (2) the freezing of side chains in the binding pocket of the IgG molecule (ΔS_{sc}), and (3) a statistical term arising from the change in the number of particles in solution (ΔS_{np}).

$$\Delta S^* = \Delta S_{pep} + \Delta S_{sc} + \Delta S_{np}. \quad (6)$$

For protein unfolding the average configurational entropy change per amino acid residue is 4.3 ± 0.1 cal K^{-1} (mol-res)$^{-1}$.[14–16] This term includes configurational entropy changes from the backbone and from the sidechains. Using this average value, ΔS_{pep} is estimated to be −34.4 ± 0.8 cal K^{-1} mol^{-1} due to the loss of degrees of freedom of the eight amino acid residues of AII.

The average value of the configurational ΔS obtained from the protein thermodynamic database does not distinguish between configurational entropies of amino acids with different sidechains. In the analysis of folding/unfolding transitions,[5,6] this does not present a serious problem because of the large number of amino acids residues involved in the transition. In the case of a small peptide however, deviations from the average can be significant. One possible way of approximating the configurational entropy per amino acid is to use available data on helix forming propensities of different amino acids. Recently O'Neil and DeGrado[17] determined the ΔG contribution of each of the 20 amino acid residues in an α-helix dimer to random coil transition using host–guest studies. They tabulated their data at 25°C as $\Delta\Delta G$ values in which the ΔG of each peptide was subtracted from the ΔG of the Gly peptide. To a first approximation, the differences in ΔG can be considered to be entirely entropic[18] and to reflect configurational entropy changes relative to Gly.

The average configurational entropy change in protein folding/unfolding transitions can be written in terms of the configurational entropy change of each amino acid type weighted by its occurrence, P_i, in globular proteins:

$$\Delta S_{avg} = \sum_i P_i (\Delta S_{Gly} - \Delta\Delta S_i). \quad (7)$$

Knowing the average value of 4.3 cal K^{-1} (mol-res)$^{-1}$, P_i and $\Delta\Delta S_i$ for each residue type, it is possible to estimate ΔS_{Gly} and subsequently ΔS for each amino acid. The results of this analysis are shown in Table II. These values represent average contributions per amino acid type and include backbone as well as sidechain contributions. The values in Table II were calculated from the O'Neil and DeGrado data except for Pro. In Table II ΔS_{Pro} is assumed to be equal to ΔS_{Ala} since the data of O'Neil and DeGrado would seem to indicate that Pro is more flexible than Gly in the unfolded state.[17]

Solving for ΔS in Eq. (7) yields a configurational ΔS value of 5.5 cal K^{-1} mol^{-1} for Gly. Using these values and the sequence of AII, a ΔS_{pep} of −35.9 ± 0.8 cal K^{-1} mol^{-1} is obtained. This value is about 7% more negative but within the estimated error of that obtained using the protein average. As error

TABLE II. Estimated Configurational Entropy Changes for Individual Amino Acid Residues Upon Protein Unfolding*

Residue	Occurrence (%)†	$\Delta\Delta S$	ΔS
Ala	9.0	2.6	3.0
Arg	4.7	2.3	3.2
Asn	4.4	0.2	5.3
Asp	5.5	0.5	5.0
Cys	2.8	0.8	4.8
Gln	3.9	1.1	4.4
Glu	6.2	0.9	4.6
Gly	7.5	0	5.5
His	2.1	0.2	5.3
Ile	4.6	0.8	4.8
Leu	7.5	2.1	3.4
Lys	7.0	2.2	3.4
Met	1.7	1.7	3.9
Phe	3.5	1.4	4.2
Pro	4.6	2.6	3.0
Ser	7.1	1.2	4.4
Thr	6.0	0.4	5.2
Trp	1.1	1.5	4.0
Tyr	3.5	0.6	5.0
Val	6.9	0.5	5.1

*Units are cal K^{-1} mol^{-1}. The values of $\Delta\Delta S$ are relative to Gly and are calculated from the $\Delta\Delta G$ values of O'Neil and DeGrado[17] assuming no enthalpic contribution. The value of Pro was taken as equal to that of Ala as described in the text.
†Values from Klapper, M.H. The independent distribution of amino acid near neighbor pairs into polypeptides. Biochem. Biophys. Res. Commun. 78:1018–1024, 1977.

estimates are not available on the O'Neil and DeGrado data, the error is assumed to be comparable to that of ΔS_{avg}. Regardless of the calculation used, similar values are calculated for the configurational entropy loss of the peptide upon binding. At 30°C the free energy resulting from the peptide configurational ΔS is estimated to be 10.4–10.9 kcal mol^{-1}.

Estimation of the entropic contribution of side chain freezing in the IgG molecule is more problematic. Recently Creamer and Rose[18] have performed Monte Carlo calculations in order to estimate the side chain entropies of amino acids in the α-helix and random coil conformations. Their results yield an average side change entropy of 2.8 cal K^{-1} mol^{-1} for the α-helix. If it is assumed that the side chains on the IgG molecule have similar rotational restrictions to residues in an α-helix and that those side chains that establish direct van der Waals contacts with AII lose their rotational degrees of freedom, then an entropy loss of approximately −2.8 cal K^{-1} mol^{-1} per side chain is expected. This assumption underestimates somewhat the configurational entropy of the IgG side chains in the binding pocket; however the terms that are neglected are mostly vibrational terms that are expected to contribute very little (<0.2 cal K^{-1} mol^{-1}). There are fourteen residues on the IgG that make van der Waals contact with AII; six of these residues make the major contribution to the interaction and are expected to be immobilized by the association.[1] Using these numbers we can estimate a ΔS_{sc} of −16.8 cal K^{-1} mol^{-1} (−2.8 × 6).

As noted above, the binding of a ligand to a macromolecule is accompanied by an additional entropy loss due to the reduction in the number of particles in solution. Since each IgG molecule binds two angiotensin II molecules, ΔS_{np} per site is given as 1/2 R ln(1/3) or −1.1 cal K^{-1} mol^{-1}.

The solvent contribution to the entropy change can be calculated from the heat capacity change as ΔC_p ln(T/T_S*). At 30°C this contribution is calculated as 61 ± 7 cal K^{-1} mol^{-1}. It should be noted that this favorable solvent contribution more than compensates the unfavorable entropy of fixing the peptide. The solvent contribution results not only from the release of solvent from the peptide, but also from the release of solvent from the binding site itself. It should be noted that if there is some marginal structure in the uncomplexed AII, the effects of this structure on configurational and solvent entropy terms will somewhat cancel each other.

The estimated value of ΔS*, including ΔS_{pep}, ΔS_{sc}, and ΔS_{np}, is −52 ± 3 cal K^{-1} mol^{-1} if ΔS_{avg} is used in calculating ΔS_{pep}, or −54 ± 3 cal K^{-1} mol^{-1} if the ΔS values from Table II are used. The overall ΔS at 30°C is therefore estimated as 7 or 9 cal K^{-1} mol^{-1}. This is again in reasonable agreement with the experimental value of 6.9 cal K^{-1} mol^{-1}; however the final error in the estimate of ΔS is around 8 cal K^{-1} mol^{-1}.

Free energy changes

The binding affinity is determined by the Gibbs free energy change which is a function of the enthalpy and entropy changes:

$$\Delta G = \Delta H - T\Delta S. \quad (8)$$

At 30°C the theoretical value for ΔG is −11.0 ± 0.5 kcal mol^{-1}, or −10.6 ± 0.5 using the ΔS values from Table II, again in reasonable agreement with the experimentally determined value of −11.0 to −11.8 kcal mol^{-1}. The results of the structural thermodynamic calculations are summarized in Table III.

Several strategies aimed at calculating the energetics of protein/protein or protein/ligand associations based on crystallographic structures have been published.[12,19] The approach utilized here in analyzing the structural energetics of AII binding to the antibody differs in several respects from previous approaches. The primary difference is that the structural parametrization of the energetics has been performed at the level of ΔH, ΔS, and ΔC_p rather than ΔG itself, since not all the terms that contribute to ΔG scale with the accessible surface area. It has been shown experimentally that ΔC_p is directly

TABLE III. Calculated Energetics of Angiotensin II Binding to IgG

Change in apolar area = -992.9 Å^2
Change in polar area = -744.8 Å^2
$\Delta C_p = -250$ cal K^{-1} mol^{-1}
$\Delta H^* = -26$ kcal mol^{-1}
$\Delta S^* = -52$ cal K^{-1} mol^{-1}
At 30°C
$\Delta H = -8.4$ kcal mol^{-1}
$\Delta S = 7$ cal K^{-1} mol^{-1}
$\Delta G = -11$ kcal mol^{-1}

related to the solvent accessible polar and apolar surface in both model compounds and proteins[5,6,8,9,20] and that ΔH for proteins can also be parameterized in terms of these quantities.[5,6] The situation is different with the entropy change. In general, the entropy can be dissected into a purely statistical term (ΔS^*) and a solvent dependent term. Only the solvent dependent term is a direct function of the change in accessible polar and apolar surface areas through its dependence on ΔC_p. The statistical term contains contributions arising from changes in the degrees of freedom of the peptide backbone and side chains that do not scale in terms of accessible surface areas. In addition, the contribution of other effects like protonation/deprotonation events need to be treated individually. Finally, by parametrizing the fundamental components of ΔG, it is possible to calculate the binding energetics as a function of temperature which is not possible with techniques which only deal with ΔG.

CONCLUSIONS

The results presented here provide an example of how flexible polypeptide hormones can form tight binding complexes with their receptors in spite of the entropic costs inherent to the binding process. The calculations indicate that the combined loss of configurational entropy resulting from the fixing of the hormone and of the sidechains in the binding pocket (-53.8 cal K^{-1} mol^{-1}) costs 16.3 kcal mol^{-1} in free energy at 30°C. This is completely offset, however, by the gain in entropy from release of solvent (61 cal K^{-1} mol^{-1}) which favors binding by -18.5 kcal mol^{-1}. The net result is that binding is moderately favored entropically by about -2.6 kcal mol^{-1}. The major contribution to the tight binding is thus enthalpic in origin. The structural energetic calculations indicate that at 30°C the burial of polar surface (i.e., hydrogen bonding) favors binding by -39.7 kcal mol^{-1} but that this is somewhat mitigated by the enthalpically unfavorable burial of apolar surface (31.3 kcal mol^{-1}). Finally, the proton released by the complex contributes about -3 kcal mol^{-1} to the overall binding energetics in phosphate buffer at pH 7. Thus the problem of binding a flexible polypeptide hormone to a receptor is solved in much the same way as the folding of a flexible polypeptide chain into a globular protein. The configurational entropy cost is offset by the release of structured water around hydrophobic groups, and the overall free energy difference results from small differences in large competing effects.

The agreement between the experimentally determined energetics for the interaction of angiotensin II with its antibody and the structural thermodynamic calculations based on the crystal structure of the complex indicates the feasibility of performing quantitatively accurate predictions of protein–ligand energetics using an empirically derived set of fundamental thermodynamic parameters. Rational protein and drug design strategies should benefit from this kind of approach.

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