Association Entropy in Adsorption Processes

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ABSTRACT The association of two species to form a bound complex, e.g., the binding of a ligand to a protein or the adsorption of a peptide on a lipid membrane, involves an entropy loss, reflecting the conversion of free translational and rotational degrees of freedom into bound motions. Previous theoretical estimates of the standard entropy change in bimolecular binding processes, ΔS°, have been derived from the root-mean-square fluctuations in protein crystals, suggesting $\Delta S^\circ \approx -50$ e.u., i.e., $T\Delta S^\circ \approx -25\ kT = -15$ kcal/mol. In this work we focus on adsorption, rather than binding processes. We first present a simple statistical-thermodynamic scheme for calculating the adsorption entropy, including its resolution into translational and rotational contributions, using the known distance-orientation dependent binding (adsorption) potential. We then utilize this scheme to calculate the free energy of interaction and entropy of pentalysine adsorption onto a lipid membrane, obtaining $T\Delta S^\circ \approx -1.7\ kT \approx -1.3$ kcal/mol. Most of this entropy change is due to the conversion of one free translation into a bound motion, the rest arising from the confinement of two rotational degrees of freedom. The smaller entropy loss in adsorption compared to binding processes arises partly because a smaller number of degrees of freedom become restricted, but mainly due to the fact that the binding potential is much "softer."

INTRODUCTION

The association of two freely translating and rotating molecules to form a complex, in the gas phase or in solution, involves a loss of entropy. Therefore, the complex will survive as a stable species only if its formation is favored on enthalpic grounds. That is, the potential energy of the bound complex must be lower than that of the well-separated molecules. In solution it is the potential of mean force that must be lower.

In biological systems one is usually interested in the association of two polyatomic species, e.g., a drug molecule binding to a protein or DNA, a ligand binding to a receptor, or a protein adsorbing on a lipid membrane. The complex is typically noncovalently bound, with binding free energies on the order of $-15\ kT$. Even if the internal degrees of freedom (vibrations and internal rotations) of the bound pair are not affected by the association process, there are usually six degrees of freedom that are affected by the formation of the complex. Three of these are the relative translational motions of the two species; center of mass translations play no role in the process. The other three (of a total of six) are rotational degrees of freedom corresponding to their relative orientation; three other rotational degrees of freedom, corresponding to the overall rotations of the complex, do not affect the association.

Upon complex formation these six degrees of freedom convert into "oscillations" (vibrations and/or hindered rotations) within the potential well of the complex. In general, these oscillations are "soft," i.e., their frequencies are low, with average energies per mode on the order of kT, where k is Boltzmann's constant and T the absolute temperature. In some systems, certain degrees of freedom may remain "free" even in the complex, e.g., a rotation of an elongated ligand around its long axis, or the two lateral translations of a molecule adsorbed on the surface of a membrane. Assuming, as we do in this paper, that the bound motions are indeed soft, they are adequately described by classical statistical thermodynamics. The classical limit is certainly adequate for describing the free motions of the separated species. This in turn implies that momentum factors (integrals) appearing in the molecular partition functions of the separated species and the complex are totally irrelevant in the statistical thermodynamic description of the association process. They cancel out identically in all the expressions (equilibrium constants) governing the association equilibrium (Mayer and Mayer, 1946; Erickson, 1979, 1989; Finkelstein and Janin, 1989; Holtzer, 1995; Janin, 1995; Ben-Shaul et al., 1996; Brady and Sharp, 1997a; Gilson et al., 1997). One only needs to consider contributions from the configurational partition functions of these molecules.

The configurational space of the separated, independently translating and rotating, species is $\Omega = (8\pi^2 V)^2$. One $8\pi^2$ factor for each species arises from the integral over the three (Euler) angles describing their orientation. Similarly, one volume factor V arises from the integral over the translational coordinates. The configurational space of the associated pair can be written as $\delta\Omega_b = (8\pi^2 V)\delta\Upsilon\delta V$. The first factor accounts for the overall translation and rotation of the complex, whereas $\delta\Upsilon\delta V$ represents the (6-dimensional) phase space volume corresponding to the relative motions (oscillations) of the two species with respect to each other within the complex. We refer to $\delta\Upsilon$ as the (restricted)

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rotational "volume" and to δV as the restricted spatial volume of the bound motions. Although the internal—angular and positional—degrees of freedom are generally inseparable, it is clear that the decomposition $\delta\Omega = \delta\Omega_b/(8\pi^2 V) = \delta\Upsilon\delta V$ is dimensionally correct, that is, it must have the dimensions of [length]^3. One may interpret $(\delta\Upsilon)^{1/3}$ as the average angular amplitude of the hindered rotations, and $(\delta V)^{1/3}$ as that of the bound spatial oscillations (see, e.g., Hill (1985)).

The entropy change attendant upon the association of the initially separated species to form a complex is $\Delta S = k \ln(\delta\Omega/\Omega) = k \ln(\delta\Upsilon/8\pi^2) + k \ln(\delta V/V) \equiv \Delta S_{rot} + \Delta S_{trans}$, with ΔS_{rot} and ΔS_{trans} representing the rotational and translational entropy losses, respectively (Erickson, 1979, 1989; Finkelstein and Janin, 1989; Janin, 1995; Gilson et al., 1997; Brady and Sharp, 1997a). Both quantities are negative since $\delta\Upsilon/(8\pi^2) \ll 1$ and $\delta V/V \ll 1$. The "standard" entropy change is specified once we define the "standard volume," e.g., $V = V_o = 1000\ \text{cm}^3/(6.023 \times 10^{23}) \cong 1660\ \text{Å}^3$ when the standard state corresponds to 1 M.

All the above notions are intuitively obvious, have been cast in simple terms with the help of elementary statistical thermodynamics (e.g., Hill, 1985; Holtzer, 1995; Janin, 1996; Gilson et al., 1997; Brady and Sharp, 1997a), and have been proved useful in the analysis of complex formation in many biological systems (Steinberg and Scheraga, 1963; Go and Scheraga, 1969; Page and Jencks, 1971; Jencks, 1975; Chothia et al., 1976; Janin and Chothia, 1978; Erickson, 1979, 1989; Dwyer and Bloomfield, 1981; Finkelstein and Janin, 1989; Novotny et al., 1989; Horton and Lewis, 1992; Searle and Williams, 1992; Searle et al., 1992; Peitzsch and McLaughlin, 1993; Murphy et al., 1994; Tidor and Karplus, 1994; Holtzer, 1995; Janin, 1995; Morton et al., 1995; Ben-Shaul et al., 1996; Brady and Sharp, 1997b; Froloff et al., 1997).

The vast majority of the biological systems that have been studied involve binding; there is only one study of the association entropy in partitioning processes (Peitzsch and McLaughlin, 1993) and no study of the entropy loss in adsorption processes. In the following we report an estimate of the entropy change in the adsorption process. Most theoretical and semiempirical methodologies for evaluating free energies of association are based on calculating the contribution of the potential of mean force and adding an estimate of the entropy (e.g., Novotny et al., 1989; Vajda et al., 1994; Weng et al., 1996). The main objective of this manuscript is to report detailed calculations of the association entropy for the adsorption of peptides on membranes, which are (to the best of our knowledge) the first ones in a biological system.

Many proteins contain clusters of basic amino acids (e.g., a sequence of five lysine residues), which facilitate membrane-association through electrostatic interactions with acidic membrane lipids (e.g., Murray et al., 1997). We have studied the membrane association of two types of model systems: positively charged peptides, such as pentalysine (Ben-Tal et al., 1996; Murray et al., 1998, 1999), and small positively charged proteins, such as charybdotoxin (Ben-Tal et al., 1997). We have used classical electrostatics in the framework of continuum solvent models and calculated the surface excess concentration of the peptides/toxins near lipid bilayers of different compositions of negatively charged 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoserine (PS) and neutrally charged 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (PC) lipids. In this paper we analyze the relative enthalpy and association entropy components of our data on the adsorption of pentalysine onto 2:1 PC/PS membrane.

ADSORPTION THERMODYNAMICS

In this section we review the thermodynamics of adsorption. Some of the derivations presented below appear in textbooks (e.g., Hill, 1985; Adamson, 1990) and in review articles (e.g., Gilson et al., 1997). We include these derivations for completeness and to avoid ambiguities regarding the choice of standard states (e.g., for partitioning coefficient versus surface excess; White et al., 1998).

Consider the adsorption of a peptide, P, onto the surface of a lipid membrane. Let A denote the total surface area available for adsorption, e.g., the outer surface of a large (and hence essentially planar) vesicle. Identifying the xy plane with the membrane surface we assume that the binding (adsorption) potential $W(\mathbf{r}, \Upsilon)$ depends only on the normal distance of the peptide from the surface, $W(\mathbf{r}, \Upsilon) = W(z, \Upsilon)$, and the relative orientation of the peptide with respect to the membrane, Υ, as specified by three Euler angles. The binding potential is chosen so that $W(z, \Upsilon) = 0$ as $z \to \infty$. More specifically, we assume that the binding potential is of finite range, λ, so that $W(z, \Upsilon) \equiv 0$ for $z > \lambda$, i.e., the peptide is considered as "bound" if $z \leq \lambda$. It should be noted that because the adsorption process takes place in solution, $W(z, \Upsilon) = W(z, \Upsilon; T)$ is in fact a potential of mean force, representing the thermal average of the adsorbate-surface interaction potential over the solvent's degrees of freedom. In the theoretical analysis and the calculations presented below, the solvent is treated as a continuous medium. Within this scheme one can account (approximately) for the fact that the adsorption potential is, actually, an interaction *free energy* (i.e., potential of mean force), by allowing the solvent's properties (e.g., the dielectric constant) to vary with temperature. However, all the calculations presented below refer to one specific (room) temperature and do not involve any temperature derivatives. Thus, the (generally very weak) dependence of W on T does not enter our calculations and hence, throughout the discussion, we shall treat W as a temperature-independent quantity.

Upon binding, four degrees of freedom (three rotations and the translation along z) become bound motions. If the peptide is symmetric with respect to rotations around its

axis, only two of the three rotations change their character upon binding; this is the case considered in the numerical calculations presented in the next section.

Consider now an aqueous solution of volume V containing, say, one giant vesicle, of adsorption area A and $N = N_f + N_b$ peptide molecules, N_f and N_b denoting the numbers of free and bound peptides, respectively. Assuming dilute solution behavior (i.e., the peptides to not interact with each other, neither in solution nor in the adsorbed state) the equilibrium ratio N_b/N_f is given by (Hill, 1986),

$$\frac{N_b}{N_f} = \frac{q_b V_b}{q_f V_f} \cong \frac{\lambda A}{V}\frac{q_b}{q_f} \tag{1}$$

where $V_b = \lambda A$ is the binding volume and $V_f \simeq V$ is the free volume. q_b and q_f are the configurational partition functions of the peptide, per unit volume, in the bound and free states. More explicitly

$$q_b = \frac{1}{V_b}\int d\mathbf{r}d\Upsilon \exp[-\beta W(\mathbf{r}, \Upsilon)] = \frac{1}{\lambda}\int_0^\lambda dz q(z) \tag{2}$$

where $\beta = 1/(kT)$ and

$$q(z) = \int d\Upsilon \exp[-\beta W(z, \Upsilon)] \tag{3}$$

is the local partition function of the peptide at distance z from the membrane surface. More precisely, $q(z)$ is a local configurational-rotational partition function. If $W \equiv 0$, as is the case for a free peptide, $q(z) = q_f = 8\pi^2$.
The quantity

$$f(z) = -kT \ln q(z) \tag{4}$$

is the configurational part of the rotational free energy, per molecule, at distance z from the surface. The entropic and energetic contributions corresponding to $f(z) = w(z) - Ts(z)$ are $w(z) = kT^2\partial \ln q(z)/\partial T = \langle W(z, \Upsilon)\rangle_\Upsilon$ and $s(z) = k \ln q(z) + kT\partial \ln q(z)/\partial T$, where $\langle W(z, \Upsilon)\rangle_\Upsilon$ denotes the (Boltzmann-weighted) average of $W(z, \Upsilon)$ over the orientations, Υ.
Using Eq. 1 we can define a partition or adsorption coefficient, κ, as

$$\kappa \equiv \frac{q_b}{q_f} = \frac{\sigma_b}{\rho_f} \tag{5}$$

where $\rho_f = N_f/V$ is the number density of free peptides in solution and $\sigma_b = N_b/V_b$ is an "effective" (3D) density of bound peptides, defined with respect to the binding volume. Alternatively, $\lambda_b\sigma_b = N_b/A$ can be regarded as the surface density of adsorbed peptides. In fact, Eq. 5, i.e., $\sigma_b = \kappa\rho_f$, is Henry's law, or the limiting form of Langmuir's adsorption isotherm at low surface coverage; it follows immediately from the requirement $\mu_b = \mu_f$, expressing the equality of the peptides' chemical potential in the adsorbed (surface) and free (bulk) phases. Explicitly, in the limit of low ρ_f and low σ_b, one has $\mu_f = \mu_f^\ominus + kT \ln \rho_f$ with $\mu_f^\ominus = -kT \ln q_f$ and similarly $\mu_b = \mu_b^\ominus + kT \ln \sigma_b$ with $\mu_b^\ominus = -kT \ln q_b$, implying $\kappa = q_b/q_f = \exp[-\beta\Delta G^\ominus]$ with $\Delta G^\ominus = \mu_b^\ominus - \mu_f^\ominus$. We use $\mu_b^\ominus$ for the "standard chemical potential" of the bound peptide to emphasize that it is defined with respect to the bound, rather than the free, volume; i.e., $\mu_b^\ominus$ is the value of μ_b corresponding to $\sigma_b = N_b/V_b = 1$, as opposed to $\mu_f^\ominus$, which corresponds to μ_f for $\rho_f = N_f/V = 1$.

The adsorption coefficient κ is a dimensionless quantity, depending only on molecular characteristics, namely on $W(z, \Upsilon)$, independent of the choice of standard state. κ is intimately related to another common quantity in adsorption thermodynamics, the surface excess concentration, Γ, defined as (e.g., Bockris and Kahn, 1993)

$$\Gamma = \int_0^\infty dz[\rho(z) - \rho_f] = \lambda\rho_f[\kappa - 1] \tag{6}$$

Here $\rho(z) = (\rho_f/q_f)\int d\Upsilon \exp[-\beta W(z, \Upsilon)] = \rho_f q(z)/q_f$ is the local concentration of peptides at distance z from the adsorbing surface. The second equality follows from our assumption that $W(z, \Upsilon)$ vanishes for $z > \lambda$.

The quantity $\Delta G^\ominus = \mu_b^\ominus - \mu_f^\ominus = -kT \ln(q_b/q_f)$ can be interpreted as the standard free energy change in adsorption. It can be decomposed into enthalpic and entropic contributions, $\Delta G^\ominus = \Delta H^\ominus - T\Delta S^\ominus$. Explicitly, the enthalpic term is,

$$\Delta H^\ominus = \frac{\int_0^\lambda dz w(z)q(z)}{\int_0^\lambda dz q(z)} \equiv \langle w(z)\rangle_b \tag{7}$$

where

$$w(z) = \frac{\int d\Upsilon W(z, \Upsilon)\exp[-\beta W(z, \Upsilon)]}{q(z)} \tag{8}$$

is the, orientationally averaged, local potential at distance z from the surface; $\langle w(z)\rangle_b$ denotes the (Boltzmann-weighted) average of $w(z)$ in the bound state. For the entropy change we have,

$$T\Delta S^\ominus = kT \ln\left[\frac{1}{q_f\lambda}\int_0^\lambda dz q(z)\right] + \langle w(z)\rangle_b \tag{9}$$

The distance-orientation dependence of $W(z, \Upsilon)$ describing the adsorption of pentalysine on a lipid membrane has been calculated recently (Ben-Tal et al., 1996). The entropy and enthalpy changes in this process will later be calculated using this $W(z, \Upsilon)$ in Eqs. 7 and 9.

In the Discussion our calculated values of $\Delta S^\ominus$ (for pentalysine adsorption on acidic membranes) will be compared to some estimates of the entropy loss in bimolecular binding processes, ΔS^o. The comparison is not entirely straightfor-

ward because, thermodynamically, the adsorption of ligands onto surfaces is a phase transition rather than a chemical reaction. A meaningful comparison is nevertheless possible, because in the limit of small concentrations of ligands both adsorption and bimolecular association processes can be treated as unimolecular reactions in which the free and bound ligands are treated as different "isomeric states" of the same molecule.

Consider first the adsorption process. Treating the bound and free ligands as two isomers, the corresponding unimolecular reaction constant, K_{uni}, can be defined using $K_{uni} = (N_b/V)/(N_f/V)$, where it should be noted that the free and bound ligand concentrations are defined with respect to the same volume, $V = V_b + V_f$: namely, the total volume of the solution. Using Eqs. 1 and 5 it follows that

$$K_{uni} = \kappa(V_b/V_f) \equiv \exp(-\Delta G^o/kT) \tag{10}$$

with the last equation serving as the definition of the (unimolecular) reaction free energy. (To become a "standard" free energy change we still need to specify the standard volumes, V_f and V_b; see below.) Then, because $\Delta G^{\ominus} = -kT \ln \kappa$, it follows that $\Delta G^o = \Delta G^{\ominus} - kT \ln(V_b/V_f)$. The second term is purely entropic, implying

$$\Delta S^o = \Delta S^{\ominus} + kT \ln(V_b/V_f) \tag{11}$$

where ΔS^o is the entropic contribution to ΔG^o, ($\Delta H^{\ominus} = \Delta H^o$).

The second term in the last equation accounts for the entropy change associated with bringing a free ligand into the confine of the binding site. It depends, of course, on the values of V_b and V_f. Once "standard" values are chosen for these volumes, ΔS^o becomes the standard entropy change in the reaction (in the limit of small ligand concentration). Momentarily postponing the choice of the standard volumes, it should be noted that in true unimolecular reactions (e.g., molecular isomerization in solution or in the gas phase), $V_b = V_f = V$ and the choice of a standard state is irrelevant; the entropy change in the reaction is independent of the choice of the standard state and is given by the first term in Eq. 11, i.e., $\Delta S^o = \Delta S^{\ominus}$. Indeed, in unimolecular processes the reaction entropy depends only on internal molecular properties, such as the stiffness of the intramolecular potentials, but is independent of the difference in zero point energies of the isomers or their available volumes (Hill, 1986). Similarly, in the adsorption process $\Delta S^{\ominus}$ depends only on the shape of the binding potential, but not on the depth of its minimum, or the volumes ascribed to the bound and free states. (In the free state the potential is flat, by definition.) To emphasize this point we note, using Eqs. 7 and 9, that if $W(z, \Upsilon)$ is also "flat" (i.e., constant within the binding region, $0 < z < \lambda$), say $W(z, \Upsilon) = -\epsilon$, then $\Delta S^{\ominus} \equiv 0$ and $\Delta H^{\ominus} = -\epsilon$ (Hill, 1985). Thus, the value of $\Delta S^{\ominus}$ reflects the deviation of $W(z, \Upsilon)$ from the behavior corresponding to a flat ("square well") potential.

With V_b and V_f describing the volumes available to the bound and free ligand, respectively, Eq. 11 can also be used to estimate entropy losses in bimolecular binding processes. Assuming that the binding site is a three-dimensional "box" of volume $V_b = \delta V = \delta x \delta y \delta z$ within which the potential energy is constant, it follows (by extension of the arguments given above) that $\Delta S^{\ominus} = 0$ and hence, $\Delta S^o = k \ln(\delta V/V_o)$, where we have set $V_f = V_o$, to emphasize that the definition of the standard entropy change, ΔS^o, requires a specification of the standard volume for the free ligands. Indeed, standard entropy changes in bimolecular binding processes have been estimated based on the above equation, $\Delta S^o = k \ln(\delta V/V_o)$, with δV estimated from the root-mean-square (rms) fluctuations of the bound ligand and $V_o = 1660$ Å^3, corresponding to a standard free ligand concentration of 1 M (see, e.g., Finkelstein and Janin (1989)). This approximate scheme can easily be correlated with our approach, which is based on Eq. 11 and involves an exact numerical calculation of $\Delta S^{\ominus}$. Further details are given in the Discussion. First, however, in the next section we elaborate on the resolution of $\Delta S^{\ominus}$ to translational and orientational contributions.

Translational and orientational entropy changes

In the next section we present a detailed calculation of $\Delta G^{\ominus}$, $\Delta S^{\ominus}$, and $\Delta H^{\ominus}$ for one special case: pentalysine adsorption on a lipid membrane. In this system, three degrees of freedom become restricted upon association: the translation normal to the membrane plane and two of the peptide rotations. Once a complex is formed, we can no longer identify one degree of freedom as a restricted translation (vibration) and the other two as hindered rotations. Nevertheless, the following scheme allows not only a calculation of the total entropy change, $\Delta S^{\ominus}$, but also an estimate of the rotational and translational contributions to this quantity.

Let $\mathcal{P}(z, \Upsilon)dzd\Upsilon$ denote the probability of finding the ligand at distance z (within z, $z + dz$) from the adsorbing surface and at orientation Υ with respect to a fixed system of coordinates attached to the surface. Outside the binding region $\mathcal{P}(z, \Upsilon)$ is uniform, i.e., it is a constant independent of z or Υ. Within the binding region

$$\begin{aligned}\mathcal{P}(z, \Upsilon) &= \frac{\exp[-\beta W(z, \Upsilon)]}{\int_o^\lambda dz \int d\Upsilon \exp[-\beta W(z, \Upsilon)]} \\ &= \frac{\exp[-\beta W(z, \Upsilon)]}{\int_o^\lambda dz q(z)} \\ &= \frac{q(z)}{\int_o^\lambda dz q(z)} \frac{\exp[-\beta W(z, \Upsilon)]}{q(z)} \\ &= \mathcal{P}(z)\mathcal{P}(\Upsilon|z)\end{aligned} \tag{12}$$

In the last equality we have expressed the joint distribution $\mathcal{P}(z, \Upsilon)$ as a product of the (marginal) distribution $\mathcal{P}(z) =$

$\int d\Upsilon \mathcal{P}(z, \Upsilon)$ and the conditional distribution $\mathcal{P}(\Upsilon|z)$. $\mathcal{P}(\Upsilon|z)$ is the probability density of finding the peptide in orientation Υ, given that its distance from the surface is z.

For a spatially and orientationally uniform distribution, $\mathcal{P}(z, \Upsilon) = \mathcal{P}_0(z, \Upsilon) = 1/(8\pi^2\lambda)$, the entropy change $\Delta S \equiv 0$. Using the familiar "$\mathcal{P} \ln \mathcal{P}$" representation of the entropy, we can express $\Delta S^{\ominus}$ in the form

$$\Delta S^{\ominus} = -k \int_0^{\lambda} dz \int d\Upsilon \mathcal{P}(z, \Upsilon) \ln\left[\frac{\mathcal{P}(z, \Upsilon)}{\mathcal{P}_0(z, \Upsilon)}\right]$$

$$= -k \int_0^{\lambda} dz \mathcal{P}(z) \ln\left[\frac{\mathcal{P}(z)}{\mathcal{P}_0(z)}\right] + \int dz \mathcal{P}(z) \Delta s(z)$$

$$\equiv \Delta S^{\ominus}_{\text{trans}} + \Delta S^{\ominus}_{\text{rot}} \qquad (13)$$

where $\mathcal{P}_0(z) = 1/\lambda$ is the uniform translational distribution in the bound region, and

$$\Delta s(z) = -k \int d\Upsilon \mathcal{P}(\Upsilon|z) \ln\left[\frac{\mathcal{P}(\Upsilon|z)}{\mathcal{P}_0(\Upsilon|z)}\right] \qquad (14)$$

is the local rotational entropy at z; $\mathcal{P}_0(\Upsilon|z) = 1/(8\pi^2)$ is the uniform (local) orientational distribution.

The last equality in Eq. 13 defines the translational and orientational contributions to the association entropy. It must be stressed, however, that we could also factorize $\mathcal{P}(z, \Upsilon)$ as $\mathcal{P}(z, \Upsilon) = \mathcal{P}(\Upsilon)\mathcal{P}(z|\Upsilon)$, in which case we would get a different decomposition of $\Delta S^{\ominus}$, which can also be interpreted as a sum of rotational and translational terms. The results obtained for the rotational and translational entropies corresponding to these different factorizations of $\mathcal{P}(z, \Upsilon)$ are generally different. They are the same only if $\mathcal{P}(z, \Upsilon) = \mathcal{P}(z)\mathcal{P}(\Upsilon)$, i.e., if the two degrees of freedom are fully independent of each other. As long as they are coupled the decomposition cannot be unique.

PENTALYSINE ADSORPTION ONTO A LIPID BILAYER

In this section we present a detailed calculation of the entropy and enthalpy changes in the course of pentalysine adsorption onto a lipid membrane composed of a 2:1 POPC/POPS mixture, in 100 mM salt solution. The interaction potential corresponding to this system has been calculated in atomic detail, taking into account the distance and orientation of the peptide with respect to the membrane surface and the corrugation of the adsorbing membrane (Ben-Tal et al., 1996). Here, however, we treat the membrane as a perfectly flat surface, that is, we average $W(x, y, z, \Upsilon)$ over the lateral coordinates x, y, obtaining the distance-orientation interaction potential $W(z, \Upsilon)$; z denoting the normal distance between the van der Waals surfaces of the peptide and the membrane when the peptide plane is parallel to the membrane plane.

The orientation of the peptide relative to the surface is specified by three angles, (χ, η, γ). χ and η were defined relative to an orientation where the peptide is parallel to the membrane surface, with its backbone along the x axis (Fig. 2 in Ben-Tal et al., 1996). Specifically, χ denotes the angle of rotation of the peptide around the z axis, i.e., around the membrane normal; η is the angle of rotation of the peptide around x, that is, around the peptide backbone; γ is the angle of rotation of the peptide around y, an axis parallel to the membrane surface and perpendicular to the peptide backbone. Assuming that the association is due only to electrostatic interactions, we identify the electrostatic free energy with $W(z, \Upsilon)$ above.

The pentalysine-membrane interaction potential has been calculated using a continuum solvent model for the electrostatic free energy. More specifically, the interaction free energy has been evaluated for (the same) 67 configurations, Υ, for closely spaced distances z within the range of the attractive potential $0 < z < \lambda$. Beyond $\lambda \approx 14$ Å the potential is practically zero; the Debye screening length corresponding to 100 mM salt solution is $l_D \approx 10$ Å.

Fig. 1 shows $w(z)$, $\Delta f(z) \equiv f(z) - f(\infty)$, and $-T\Delta s(z) = \Delta f(z) - w(z)$ for the membrane-pentalysine system; $w(z)$ is the orientationally (Υ) averaged electrostatic free energy of interaction between the peptide and the lipid bilayer in water. The Coulombic attraction between the positively charged peptide and the negatively charged membrane prevails for $z > 2.5$ Å, i.e., as long as at least one layer of water molecules separates the peptide from the bilayer (the diameter of water molecule is ≈2.8 Å). For $z \leq 2.5$ Å a portion of the region between the peptide and the membrane is no longer accessible to water and is assigned a low dielectric constant. When pentalysine approaches the membrane surface, the charges on the peptide and the membrane in this

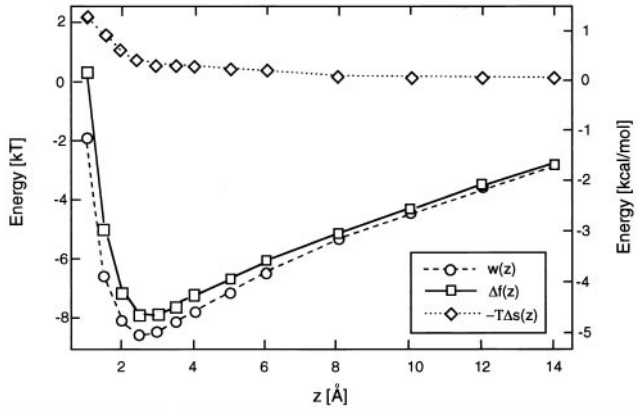

FIGURE 1 Pentalysine binding to a 2:1 PC/PS lipid bilayer in 100 mM monovalent salt solution. $\Delta f(z) \equiv f(z) - f(\infty)$ is depicted with squares connected by the solid line, $w(z)$ as circles connected by the dashed line, and $-T\Delta s(z)$ as diamonds connected by the dotted line. See text for details.

region are transferred to a low dielectric region, which gives rise to Born repulsion as explained in more detail in Ben-Tal et al. (1996) and references therein.

The calculations show that $\Delta f(z)$ is almost identical to $w(z)$ for $z > 2.5$ Å. At shorter distances, where peptide rotations are restricted, $\Delta f(z)$ becomes less negative than $w(z)$. Indeed, the difference between them, $-T\Delta s(z)$, is a monotonically decreasing function of z with a moderate slope at long distance, $z > 2.5$ Å. At shorter distances, where excluded volume constraints limit the permitted range of the tilt angles η and γ, the $-T\Delta s(z)$ curve becomes steeper. It is evident from the figure that the orientational entropy changes are significant only over a small region ($z < 2.5$ Å), smaller than the range of the attractive peptide-membrane potential, $w(z)$. We thus expect a rather small entropic contribution to the adsorption free energy.

The free energy of adsorption of pentalysine to 2:1 PC/PS lipid bilayer in 100 mM salt, calculated from the results of Fig. 1, is $\Delta G^{\ominus} = -kT \ln \kappa = -6.2\ kT$, where κ is the adsorption coefficient of Eq. 5. The enthalpic and entropic contributions to the adsorption, Eqs. 7 and 9, are $\Delta H^{\ominus} = -8.1\ kT$ and $T\Delta S^{\ominus} = -1.9\ kT$, respectively.

Decomposition using Eq. 13 shows that $\sim -1.5\ kT$ of the entropy contribution is from $\Delta S^{\ominus}_{\text{trans}}$, reflecting the restricted translational freedom of the adsorbed peptide along the membrane normal. The rest of the entropy loss, $\sim -0.4\ kT$, reflects the confinement of orientational freedom of the adsorbed peptide. We therefore estimate a free energy penalty of $\sim 1.5\ kT$ per confined translational degree of freedom, i.e., free translational degree of freedom, which becomes vibration. Again, this analysis would have been incorrect if z and Υ were strongly coupled to each other. Fig. 1 indicates that this is not the case here; $\Delta s(z)$ is almost independent of z except for very short distances, and our analysis is a reasonable approximation.

DISCUSSION

The adsorption of a peptide onto the surface of a membrane differs from "typical" bimolecular binding processes in two main respects: 1) In adsorption only one, rather than three, translational degree of freedom is converted to a bound motion. 2) The adsorbing potential is generally "softer," resulting in larger rms fluctuations of the adsorbed ligand around its equilibrium state. Both factors suggest that the entropy loss in the adsorption process should be considerably smaller than in bimolecular complex formation. In the following we compare our results from the previous sections with other relevant estimates of entropy losses in association processes. To this end we shall first need to transform our expressions for the adsorption entropy into the language commonly used to describe chemical binding equilibrium.

The translational entropy loss in adsorption, as given by the first term in Eq. 13, can be expressed in the approximate form $\Delta S^{\ominus}_{\text{trans}} \approx k \ln(\delta z/\lambda)$, where δz is the "width" of the distribution function $\mathcal{P}(z)$ within the well of the adsorption potential, and λ, as before, is the range of the potential (or, equivalently, the thickness of the layer defining the adsorption region). The width, δz, can be approximated by the rms fluctuations of the adsorbate around the minimum of the potential well, assuming that the potential is harmonic around its minimum (see below). (Alternatively, $\Delta S^{\ominus}_{\text{trans}} = k \ln(\delta z/\lambda)$ can be regarded as the definition of δz.) Similarly, $\Delta S^{\ominus}_{\text{rot}} \approx k \ln(\delta\Upsilon/8\pi^2)$, with $\delta\Upsilon$ denoting the 3D rms fluctuations in rotational angles of the bound peptide.

An identical expression to the last form of $\Delta S^{\ominus}_{\text{rot}}$, namely $\Delta S^{\text{o}}_{\text{rot}} \approx k \ln(\delta\Upsilon/8\pi^2)$, is often used to estimate the orientational entropy loss in bimolecular ligand binding processes (Erickson, 1979, 1989; Finkelstein and Janin, 1989; Janin, 1995; Gilson et al., 1997; Brady and Sharp, 1997a) (Recall that when the concentration of ligands is much smaller than that of the substrate, the bimolecular association process is, effectively, a unimolecular process with respect to the ligand. The substrate is then treated as stationary).

Typically, in bimolecular association processes, three translational degrees of freedom are converted to bound motions (as compared to one degree of freedom in the adsorption on a flat membrane). The 3D translational entropy loss in these processes is often estimated using the expression $\Delta S^{\text{o}}_{\text{trans}} \approx k \ln(\delta V/V_{\text{o}})$ (Erickson, 1979, 1989; Finkelstein and Janin, 1989; Janin, 1995; Gilson et al., 1997; Brady and Sharp, 1997a). Here, $\delta V = \delta x \delta y \delta z$ is the 3D rms fluctuation of the ligand (center of mass) position in the bound state, and $V_{\text{o}} = X_{\text{o}} Y_{\text{o}} Z_{\text{o}}$ is a reference or "standard" volume, representing the volume available to the free ligands in solution. Assuming that the bulk solution is contained in a cubic box ($X_{\text{o}} = Y_{\text{o}} = Z_{\text{o}} = V_{\text{o}}^{1/3}$), the standard translational entropy loss per one (say, the z) translational degree of freedom is $\Delta S^{\text{o,z}}_{\text{trans}} = k \ln(\delta z/V_{\text{o}}^{1/3}) \approx \Delta S^{\text{o}}_{\text{trans}}/3$.

The last expression for $\Delta S^{\text{o,z}}_{\text{trans}}$ can directly be applied to calculate the "standard" translational entropy loss in *adsorption*. Using this definition we note $\Delta S^{\text{o,z}}_{\text{trans}} = \Delta S^{\ominus}_{\text{trans}} + k \ln(\lambda/V_{\text{o}}^{1/3})$. The interpretation of this equation is straightforward. The second term on its right-hand side is the entropy change associated with bringing the ligand from (its standard state in) the bulk solution into the adsorption layer, λ. The first term reflects the entropy loss associated with the fact that once adsorbed, the center of mass position of the ligand is actually confined to a small range δz around the minimum of the potential well.

The most common choice of a standard state corresponds to a (hypothetical, ideal) solution containing free ligands at concentration of 1 M (see, for example, Finkelstein and Janin, 1989). This, in turn, implies $V_{\text{o}} \approx 1660$ Å^3 and hence $V_{\text{o}}^{1/3} \approx 11.84$ Å. (The small value of V_{o} represents the "average volume per solute particle" in a solution containing an Avogadro number of indistinguishable solutes. One should not attribute much physical significance to this

value, neither to the assumption that the solution is ideal. One could just as well choose a more realistic standard state, e.g., a 1 μM ideal solution. We shall use the 1 M standard state because this is the usual choice.)

Using the above value of V_o and our $\lambda = 14.25$ Å, we obtain $k \ln(\lambda/V_o^{1/3}) = 0.2\ k$. (Again, the apparent increase in entropy upon transferring the free solute into the adsorption layer is a consequence of the choice of a small value for V_o, the entropy change in this process would be negative if the standard volume was just slightly larger.) For the standard translational entropy change in the adsorption process we obtain $-T\Delta S^{o,z}_{trans} = -T\Delta S^{\ominus}_{trans} - kT \ln(\lambda/V_o^{1/3}) = 1.5\ kT - 0.2\ kT = 1.3\ kT$, i.e., $\Delta S^{o,z}_{trans}$ and $\Delta S^{\ominus}_{trans}$ are not very different in this case.

Before comparing the value of $\Delta S^{o,z}_{trans}$ with other estimates it is instructive to examine the approximate expression $\Delta S^{\ominus}_{trans} = k \ln(\delta z/\lambda)$, with δz measuring the rms fluctuations of the peptide in harmonic potential in z against the exact (numerical) calculation of $\Delta S^{\ominus}_{trans}$ as given by the first term in Eq. 13. The magnitude of the force constant (from $w(z = 3\ Å) = -8.46\ kT$ and $w(z = 4\ Å) = -7.73\ kT$) is $\xi = 1.5\ kT/Å^2$. The rms fluctuations in z is then $\delta z = (2\pi kT/\xi)^{1/2} = 2.1$ Å and $-T\Delta S^{\ominus} = -kT \ln(\delta z/\lambda) = -kT \ln(2.1/14.25) = 1.9\ kT$; very similar to the calculated value, 1.5 kT. The above estimate of translational entropy loss is very similar to the value predicted by Erickson (1989), allowing rms fluctuations of $(2\ Å)^3$ in bound complexes. His argument for choosing the $(2\ Å)^3$ range was that these fluctuations would seriously disrupt the van der Waals, ionic and hydrogen bonds across the interface and larger displacements would admit water and disrupt the hydrophobic bonding. The $\sim$1.5 kT estimate per confined translational degree of freedom thus appears to be characteristic of loose complexes and may be regarded as an approximate lower limit for the entropy loss upon association in biological systems.

Our calculated value of the total (translational and rotational) standard entropy loss in the adsorption process, $-T\Delta S^o = 1.9\ kT - 0.2\ kT = 1.7\ kT$, may also be compared with the estimate obtained by Peitzsch and McLaughlin (1993) based on their measurements of the partitioning of fatty acids and acylated peptides into phospholipid vesicles. Specifically, their estimate of the standard entropy loss is $-T\Delta S^o \approx 3\ kT$.

For tight binding processes, the estimate of Finkelstein and Janin (1989) based on rms fluctuations of $(0.25\ Å)^3$ taken from B factors in protein crystals seems quite reasonable. They obtain 12 kT for confining three translational degrees of freedom and about the same for the three rotations. We may regard their estimate as corresponding to an approximate upper limit. To summarize, the difference between our low estimate of $-T\Delta S^o = 1.7\ kT$ and their high estimate of $\sim$25 kT is a consequence of two reasons: 1) only three of six external degrees of freedom are confined in adsorption processes, and 2) the confined degrees of freedom are less tightly bound in adsorption than in binding processes.

Taken together, our results indicate that association entropy plays a minor, yet non-negligible, role in adsorption. Obviously, it depends on the physical dimension of the adsorbed peptide and on the depth of the binding potential; a longer and/or more tightly adsorbed peptide would be more confined, leading to a larger association entropy. In addition, the more degrees of freedom restricted in the association, the larger the association entropy.

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