The Cost of Conformational Order: Entropy Changes in Molecular Associations

Mark S. Searle and Dudley H. Williams*

Contribution from the Cambridge Centre for Molecular Recognition, University Chemical Laboratory, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK.
Received June 15, 1992

Abstract: Molecules in solution have degrees of freedom of overall translation and rotation, as well as internal rotations about single bonds, that become highly constrained when two molecules associate to form a stable complex. We have considered the thermodynamics of fusion and sublimation of hydrocarbons and polar organic molecules as instructive models for features of complex dissociation. The entropy of fusion within homologous series of alkanes, alkyl carboxylic acids, and 2-methyl ketones provides an estimate of the entropic cost of restricting a rotor, within a hydrocarbon chain, as −1.6 to −3.6 kJ mol^{-1} ($T\Delta S$ at 300 K) upon crystallization. Data on the fusion and sublimation of a range of organic substances (RMM 100–300 g mol^{-1}) that are essentially free of internal rotors illustrate the compensatory relationship between enthalpy and entropy of phase transitions that bear on the cost of restraining a freely translating and rotating molecule in forming a crystal (cf. molecular recognition complex). A comparison of calculated gas phase translational and rotational entropies with entropies of sublimation reveals that the latter can account for only 40–70% of the gas phase entropies. The difference, attributed mainly to the entropy of residual motions in the crystal, gives an insight into the favorable entropy of residual motions in molecular recognition complexes. When entropies of sublimation are corrected by Trouton's rule for condensation to a liquid, we conclude that some bimolecular associations to form complexes with little exothermicity may have relatively small adverse entropies, which may lie anywhere in the range −9 to −45 kJ mol^{-1} (in terms of $T\Delta S$ at 300 K), opposing binding by a factor of $10^{-1.6}$ to 10^{-8} M^{-1}. These "enthalpy/entropy compensations" are of general relevance to binding interactions of biological importance and are discussed with respect to the binding of agonists versus antagonists to a common receptor site.

Introduction

There is an entropic cost associated with any bimolecular interaction that is a consequence of degrees of freedom of motion lost when two molecules are rigidly constrained within a complex.[2-7] When two molecules associate, twelve degrees of freedom of motion (three each of both translation and rotation per molecule) are reduced to only six in such a hypothetically "rigid" complex. If the binding process involves capturing a flexible molecule, whose internal rotations about single bonds must also be restricted, the consequence is a further adverse entropic penalty that results in a reduction in binding constant K, according to the classical relationships

$$\Delta G = \Delta H - T\Delta S \quad \text{and} \quad \Delta G = -RT \ln K$$

If we are to begin to predict binding constants for bimolecular associations in a manner that might develop useful rules for understanding the forces that drive molecular recognition,[7] then we need to be able to semiquantitate the adverse cost in free energy of generating conformational order from associations of flexible molecules. The estimation of this cost is difficult for several reasons. First, the entropies of molecules in solution are subject to uncertainties.[2-7] Second, the amount of residual motion present in a complex formed by association in solution is also uncertain. Third, as we emphasize in a later part of this paper, this amount of residual motion in a complex may vary as a function of the nature and extent of the intermolecular forces (enthalpy term) involved in binding. Additionally, the issue is complicated by the fact that the accounting of the balance of entropy changes may be done in different ways. For example, it may be considered that the degrees of vibrational freedom associated with residual motion in the complex are translational and rotational entropy of the separate components which are not lost upon association;[5] alternatively, this vibrational freedom can be regarded as a benefit of the association, appearing after the interacting components have lost all of their translational and rotational entropy.[8] The latter approach gives rise to larger binding constants for functional group interactions than the former;[8] for example, the latter approach gives rise to estimated binding selectivities in the range 10^3–10^4 for the amide–amide hydrogen bond in aqueous solution, whereas the former approach gives smaller values in the range $10^{0.7}$–10^2, as outlined in the following paper.[9] We recommend that the former approach be generally adopted and illustrate the point by reference to an extreme example. The free energy of transfer of pentane from an aqueous environment to an environment of bulk pentane is −28 kJ mol^{-1} at 298 K.[10] Since $\Delta G = -RT \ln K$, the "binding constant" K, reflecting the preference for the hydrocarbon environment, is ca. 10^5 M^{-1}. The process is entropy driven (by the disordering of water when pentane passes from the aqueous to hydrocarbon phase), as evidenced by the very small enthalpy change (ΔH = 2 kJ mol^{-1}).[10] The transferred pentane molecules have essentially the same translational and rotational entropy before and after transfer, and the absence of stronger intermolecular forces to the pentane after transfer to bulk pentane from water is the physical justification for this approximation. It would clearly be undesirable to credit this pentane–pentane interaction for the residual motion it allows (ca. 10^9 M^{-1}), and the "binding constant" is properly taken as ca. 10^5 M^{-1} and not 10^{14} M^{-1}. However, as an association becomes increasingly exothermic, an increasing price in loss in translational and rotational entropy will be paid (up to a limit of ca. 10^{-9} M^{-1} for a relative molecular mass of ca. 100).[2,11] Subsequent research must attempt to assess a

(1) Abbreviations: ΔG, ΔH, and ΔS, change in Gibb's free energy, enthalpy, and entropy; NMR, nuclear magnetic resonance; RMM, relative molecular mass; ΔH_{fusion} and ΔS_{fusion}, enthalpy and entropy of fusion; T_m, melting temperature (fusion); ΔS^t_{t+r}, change in entropy of translation and rotation for fusion; ΔS^t_r, change in entropy of internal rotations for fusion; S_{trans}, translational entropy; S_{rot}, rotational entropy; ΔG_{t+r} and ΔS_{t+r}, change in free energy and entropy of translational and rotation; ΔH_{sub} and ΔS_{sub}, enthalpy and entropy of sublimation; ΔS^s_{t+r}, change in entropy of translation and rotation for sublimation; ΔS^s_r, change in entropy in internal rotations for sublimation; C_p, heat capacity at constant pressure; X_A and X_B, mole fraction of A and B; ΔS_{mix}, change in entropy on mixing.

(2) Page, M. I.; Jencks, W. P. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1678–1683.

(3) Jencks, W. P. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4046–4050.

(4) Jencks, W. P. *Adv. Enzymol.* **1975**, *43*, 219–410.

(5) Finkelstein, A. V.; Janin, J. *Protein Eng.* **1989**, *3*, 1–3.

(6) Page, M. I. *Chem. Soc. Rev.* **1973**, *2*, 295–323. Page, M. I. *Angew. Chem.* **1977**, *16*, 449–459.

(7) Williams, D. H.; Cox, J. P. L.; Doig, A. J.; Gardner, M.; Gerhard, U.; Kaye, P.; Lal, A.; Nicholls, I. A.; Salter, C. J.; Mitchell, R. C. *J. Am. Chem. Soc.* **1991**, *113*, 7020–7030.

(8) Doig, A. J.; Williams, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 338–343.

(9) Searle, M. S.; Williams, D. H.; Gerhard, U. *J. Am. Chem. Soc.*, following paper in this issue.

(10) Doig, A. J.; Williams, D. H. *J. Mol. Biol.* **1991**, *217*, 389–398.

0002-7863/92/1514-10690$03.00/0 © 1992 American Chemical Society

more precise relationship between these variables, in order to allow the first-mentioned approach to be applied.

In order for productive binding to occur ($\Delta G < 0$), the above adverse free energy costs in bringing about conformational order must be offset by favorable intermolecular interactions, such as hydrogen bonds, van der Waals packing, and the increased entropy associated with solvent randomization.[12] In the first part of this paper, we compare thermodynamic data for crystallization of a variety of organic molecules as a model for entropic changes in molecular recognition. The crystallization of organic molecules from a melt is characterized by entropy changes in some ways similar to those occurring in ligand/receptor interactions in solution. In the second part of this paper, we draw attention to the enthalpy/entropy compensation that occurs in the melting and sublimation of organic crystals. Although this compensation has been appreciated in thermodynamic measurements of many kinds of phenomena for a long time,[13] it has consequences for the molecular details of ligand/receptor interactions which we believe merit emphasis. In particular, we comment on the implications for the mechanisms of action of agonists versus antagonists.[14,15]

Discussion

Crystalline substances provide a model of a complex in which degrees of freedom of overall translation, rotation, and internal rotors of individual molecules are severely restricted by comparison with molecular motions in a pure liquid or in a solution. Thus, melting of organic crystals provides a model for complex dissociation. The enthalpy of fusion ΔH_{fusion} allows ΔS_{fusion} at the melting temperature T_m to be calculated from the relationship

$$\Delta S_{fusion} = \Delta H_{fusion}/T_m$$

Since there is residual motion in the crystal, there is a large entropy that remains in such a structure. However, ΔS_{fusion} reflects (i) an increase in entropy because molecular translations and rotations, together with degrees of freedom of internal bond rotations (ΔS^f_r), are increased upon melting and (ii) a decrease in entropy because residual motions/soft vibrations in the solid are lost upon melting. Since entropies of fusion are always positive, it is clear that the former effect is always greater than the latter. We factorize ΔS_{fusion} in the following manner to take account of these terms:

$$\Delta S_{fusion} = \Delta S^f_{t+r} + \Delta S^f_r \quad (1)$$

We take the view that ΔS^f_{t+r} represents the difference in entropy between relatively free motions in the melt and residual translations and rotations in the solid that are not lost in the crystallization process and ΔS^f_r represents the entropy of internal bond rotations.[16] An analogous expression may be written for the dissociation of a complex in solution. However, the corresponding terms are expected to be somewhat larger than their fusion counterparts. This follows since a melt is still ordered to some extent by the intermolecular forces that remain between neighboring molecules

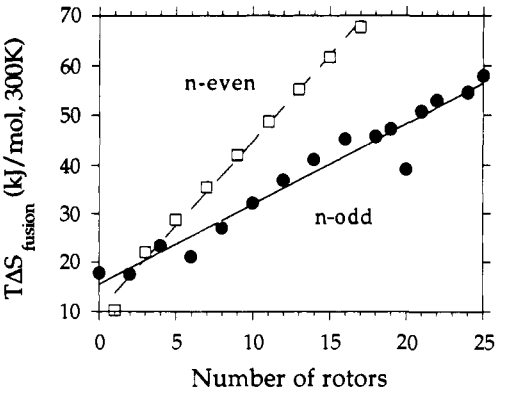
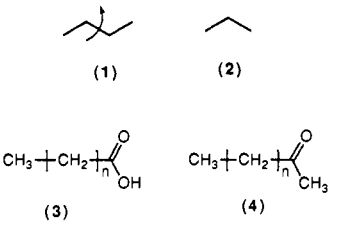


Figure 1. Plot of entropy of fusion (at 300 K) versus the number of rotations released in the melting of *n*-alkanes. Hydrocarbons with an even number of carbon atoms in the chain are plotted separately from those with an odd number (see text for details). Data are fitted to the following equations: (*n* odd), $y = 15.5 + 1.6x$ with the correlation coefficient (R) = 0.97; (*n* even), $y = 10.2 + 3.5x$ with $R = 0.99$.

Chart I



(for example, enthalpies of evaporation frequently lie in the range 2–12 times greater than enthalpies of melting;[17] in contrast, in dissociation of complexes in solution, the partners originally complexed normally become completely separated by solvent molecules). Additionally, we will normally be interested in dissociation of complexes where ΔG for dissociation is >0. The thermodynamic parameters for the fusion process are determined at T_m where $\Delta G = 0$. At temperatures less than T_m there will be less thermal motion, and therefore the crystal will be stabilized more enthalpically and less entropically than at T_m. When this effect is considered, processes with $\Delta G > 0$ will tend to be associated with larger entropy changes than the crystal model. Thus, for both these reasons, all the above terms for melting will tend to be less than the corresponding terms for complex dissociation in molecular recognition. In the following sections, we evaluate some of these terms (or combinations of them) for the melting of organic molecules. Since we are interested in comparing the values to those which might occur in molecular recognition phenomena at room temperature, the various entropies are expressed in terms of $T\Delta S$ at 300 K.

We consider first $T\Delta S_{fusion}$ values for linear hydrocarbons[18] in the homologous series C_nH_{2n+2} and plot $T\Delta S_{fusion}$ versus the number of rotors in the hydrocarbon chain that will be restricted in the crystal. For example in Chart I, butane (**1**) has a single rotor and propane (**2**) has no rotors, while in general for a C_n hydrocarbon chain $n - 3$ rotors are restricted. We emphasize the term "restricted" rather than "frozen" to indicate that relatively free rotation about a bond in solution is replaced by a torsional vibration in the crystal, which also has some entropy. We return to this theme in subsequent discussion. Methyl groups have small barriers to rotation in the crystalline environment and are regarded as freely rotating.[19] Modifying eq 1 to allow for the restriction

(11) Williams, D. H. *Aldrichim. Acta* **1991**, *24*, 71–80.

(12) We do not intend to imply in this description that increased entropy associated with solvent randomization is a "cause". The cause of any spontaneous change is of course a negative ΔG. Solvent water (for example) is randomized (entropically favorable) upon hydrocarbon burial because removal of hydrocarbon from water gets rid of water molecules at the hydrocarbon/water interface which make fewer but stronger hydrogen bonds than bulk water, and hence are more organized than bulk water. The system spontaneously optimizes bonding both before and after hydrocarbon burial, but after hydrocarbon burial this optimization of bonding is achieved at a lower cost in entropy, and hence with a negative ΔG.

(13) (a) Lemieux, R. U.; Delbaere, L. T. J.; Beierbeck, H.; Spohr, U. *Host-guest molecular interactions from chemistry to biology*; Ciba Foundation Symposium; Wiley: Chichester, pp 1991; 231–248. (b) Lumry, R.; Rajender, S. *Biopolymers* **1970**, *9*, 1125–1227.

(14) Miklavc, A.; Kocjan, D.; Mavri, J.; Koller, J.; Hadzi, D. *Biochem. Pharmacol.* **1990**, *40*, 663–669.

(15) Weiland, G. A.; Minneman, K. P.; Molinoff, P. B. *Nature* **1979**, *281*, 114–117.

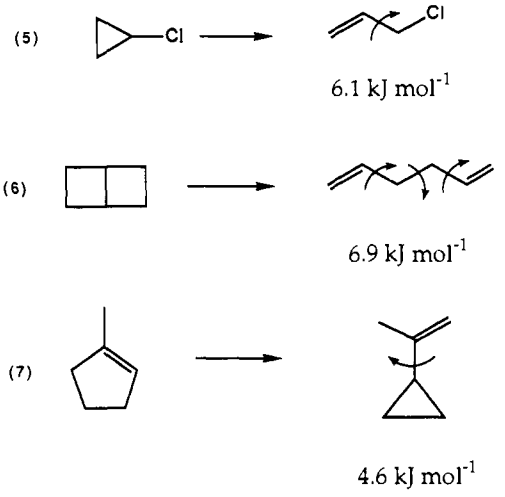
(16) In complexes held together by hydrogen bonds, for example, the intrinsic binding energy for that interaction may also be associated with some small entropic vibrational component that is an intrinsic part of the interaction rather than being residual translational and rotational entropy of the whole molecule.

(17) Dasent, W. E. *Inorganic Energetics-An Introduction*, 2nd ed.; Cambridge University Press: Cambridge, 1982; p 95.

(18) Westrum, E. F., Jr.; McCullough, J. P. Thermodynamics of crystals. In *Physics and Chemistry of the Organic Solid State*; Fox, D., Labes, M. M., Weissberger, A., Eds.; Interscience Publishers, Wiley: New York, 1963; pp 1–156.

(19) O'Reilly, D. E.; Anderson, J. H. Magnetic Properties. In *Physics and Chemistry of the Organic Solid State*; Fox, D., Labes, M. M., Weissberger, A., Eds.; Interscience Publishers, Wiley: New York, 1963; pp 202–205.

Scheme I



of n rotors at a cost of ΔS^f_r per rotor and at a temperature T we obtain

$$T\Delta S_{fusion} = T\Delta S^f_{t+r} + nT\Delta S^f_r \quad (2)$$

Plots of $T\Delta S_{fusion}$ (300 K) versus n for odd and even number carbon chains (Figure 1), considered separately, reveal good linear correlations ($R > 0.97$). These linear correlations imply that for each series of hydrocarbon chains, eq 2 simplifies to an expression of the form

$$T\Delta S_{fusion} = nT\Delta S^f_r + C \quad (3)$$

where $T\Delta S^f_{t+r}$ from eq 2 is approximately equal to a constant, given by the intercept of the straight lines with the $T\Delta S$ axis. Additionally, the slopes of the two lines give $T\Delta S^f_r$, the net cost at 300 K of restricting a single backbone rotor of the two types of linear hydrocarbon chain; these are 1.6 kJ mol^{-1} for the series with an odd number of carbons in the chain and 3.4 kJ mol^{-1} where the number of carbons is even. The distinction between the two series is necessary because both the crystal structure and the phase behavior of the even series differ from those of the odd series up to C_{20}. The even hydrocarbons from C_6 to C_{20} form triclinic crystals but show no solid–solid phase transitions. In contrast, the odd members of the series form orthorhombic crystals and do exhibit phase transitions below the melting temperature.[18] Thus, just below the melting temperature, the backbone rotors in the odd series hydrocarbons in the solid are less well ordered than those in the even series, and this is reflected in a smaller favorable entropy change per rotor on melting. Even the larger value of 3.4 kJ mol^{-1} per rotor is less than the entropic cost of "freezing" an internal rotation ($T\Delta S$ = 5–6 kJ mol^{-1} at 300 K) derived in a classic paper by Page and Jencks[2] from studies of isomerization reactions (see below). As already suggested above, some limitation of backbone motions in the melt may persist as a consequence of intermolecular interactions (e.g., if this state reflects some aspects of the limited backbone ordering of the kind found in a biological membrane). Additionally, the hydrocarbon crystals are held together by relatively weak van der Waals interactions which may leave backbone rotors with considerable entropy in the crystal as a consequence of large amplitude torsional vibrations, or even "crankshaft" motion of the hydrocarbon backbone. In contrast, Page and Jencks have considered, for example, the entropy changes for cyclization reactions involving covalent bond formation. Here, flexible molecules are transformed into more highly restrained cyclized products. The amplitude of residual torsional vibrations in the cyclic product must be considered to be more limited than those of hydrocarbon molecules weakly oriented in crystals; the consequence is a larger entropy change for the former process than the latter. To emphasize the difference between entropy changes on fusion and isomerization, several examples of the latter are illustrated in Scheme I.[20] The value for $T\Delta S$ per rotor at 300 K is obtained by dividing the total entropy change for the isomerization by the change in the number of rotors; to a first approximation, we associate the net entropy change with the increase in the number of "free" rotors in the product, which makes the forward reaction entropically favorable. Bond rotations within the cyclic starting structures are highly restricted, resulting in larger entropy changes per rotor in forming the unrestrained product than for the fusion of hydrocarbons. The largest entropy changes of between 6 and 7 kJ mol^{-1} per rotor are identified for the transformation of the small "rigid" cyclopropyl and cyclobutyl ring systems of **5** and **6**. Values of $T\Delta S$ per rotor of up to 7 kJ mol^{-1} are quite consistent with the data presented by Page and Jencks[2] on analogous molecular transformations.

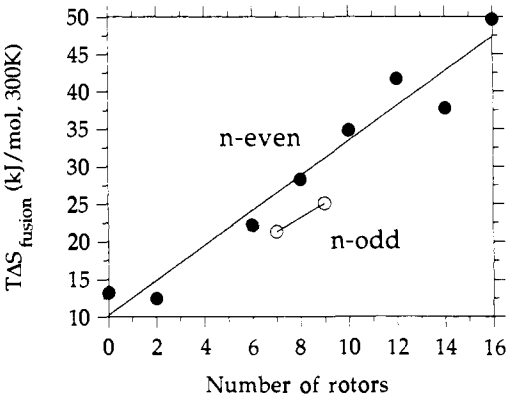


Figure 2. Plot of entropy of fusion (at 300 K) versus the number of rotations released in the melting of n-alkyl carboxylic acids. Data are fitted to the equation $y = 10.2 + 2.3x$ with $R = 0.96$ (n even).

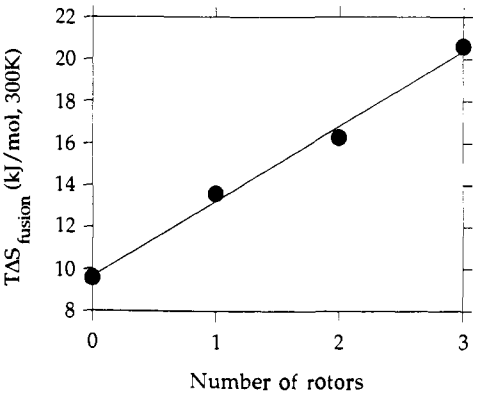


Figure 3. Plot of entropy of fusion (at 300 K) versus the number of rotations released in the melting of n-alkyl methyl ketones. Data are fitted to the equation $y = 9.7 + 3.6x$ with $R = 0.99$.

We have extended the analysis to homologous series of alkyl carboxylic acids **3** and 2-methyl ketones **4**. Similar correlations between the number of rotors and the entropy of fusion are observed (Figures 2 and 3). In these series, the "odd"/"even" differentiation is much less pronounced. The slopes of the plots indicate the average entropic cost of rotor restriction (in terms of $T\Delta S$ at 300 K) on crystal formation to be 2.3 kJ mol^{-1} (n even) for the carboxylic acids and 3.6 kJ mol^{-1} for the methyl ketones.

Our assumption from eq 2 that $T\Delta S^f_{t+r}$ is approximately constant appears to be vindicated by the good linear correlations observed in Figures 1–3; the intercepts of the lines on the $T\Delta S$ axis give values in the range −9 to −15 kJ mol^{-1} (at 300 K) for the crystallization of hydrocarbons, carboxylic acids, and methyl ketones. The physical basis for the observation that $T\Delta S^f_{t+r}$ is a constant is worthy of further comment. The translational entropy (S_{trans}) of a molecule can be estimated from the Sackur–Tetrode equation in the case of an ideal gas

$$S_{trans} = R \ln [\pi^{3/2} e^{5/2} (V/N)(2mkT/h^2)^{3/2}] \quad (4)$$

(20) Maskill, H. *The Physical Basis of Organic Chemistry*; Oxford University Press: London, 1985; pp 77–114.

For 1 mol under standard conditions (298 K) the only variables are the volume (V) and mass (m) of the molecule; the remaining terms are physical constants. The rotational entropy (S_{rot}) is described by the expression

$$S_{rot} = R \ln [(\pi^{7/2}e^{3/2})(I_A I_B I_C)^{1/2}(8kT/h^2)^{3/2}] \quad (5)$$

where I_A, I_B, and I_C are the moments of inertia about the three principal axes of the freely rotating molecule; all other terms are constants. Thus, at a given temperature, the sum of the translational and rotational entropy can be approximated to an expression of the form

$$S_{trans} + S_{rot} = R \ln (am) \quad (6)$$

where m is the mass of the molecule and a is a constant. The amplitude and entropy of molecular motions in the crystal have been considered by Finkelstein and Janin,[5] who regard them as translational and rotational motions of the individual "free" molecules that are not lost in the crystallization process. As indicated above, they can also be regarded as residual motions/vibrations that appear after all translational and rotational entropy has disappeared and can also be approximated by a similar expression to eq 6:

$$S_{trans} + S_{rot} = R \ln (bm) \quad (7)$$

where b is a constant. Therefore, the change in translational and rotational entropy (ΔS_{t+r}) is given by the expression

$$\Delta S_{t+r} = R \ln (a/b) \quad (8)$$

and becomes independent of mass and dependent only on the ratio of a and b. This ratio can be equated with the change in accessible volume available for translation and rotation per molecule in passing from the gas (or liquid) to the crystal.[5] These constants can be regarded as a property of the crystal lattice and are likely to be very similar for a given magnitude of intermolecular force (amplitude of motion) and crystal type (i.e., within either the even or odd hydrocarbon series). The important consequence is that eq 8 appears to have a fixed value for a given series of hydrocarbons, which is consistent with our conclusions that $T\Delta S^f_{t+r}$ is constant from the observation of the linear correlations observed in Figures 1–3.

Finkelstein and Janin[5] have estimated the change in volume of translation per molecule based upon the amplitude of relative movements in crystals. They point out that X-ray scattering experiments indicate whole-molecule motions of 0.2–0.25 Å in protein crystals, which they equated with $T\Delta S_{t+r}$ values at 300 K that are approximately half of the calculated gas-phase entropies, TS_{t+r}. The experimental values of $T\Delta S^f_{t+r}$ of 9 to 15 kJ mol^{-1} from the hydrocarbon crystallization data, corresponding to the intercepts of the plots in Figures 1–3, are somewhat lower than half of the calculated change in entropy of translation and rotation in solution $T\Delta S_{t+r}$ (at 300 K).[7] After considering the entropies of fusion of a variety of organic compounds (those presented in Figure 4), in the molecular mass range of ca. 100 to a few hundred daltons, that are essentially free of internal rotors, we can arrive at an estimated value for the loss of translational and rotational entropy as a function of exothermicity based on this model. For example, for a bimolecular association involving weak interactions which are exothermic in the range 20–35 kJ mol^{-1}, the model suggests loss of translational and rotational entropy ($T\Delta S$, 300 K) in the range 17–27 kJ mol^{-1}. The upper limit for this last value is close to half of the calculated value for $T\Delta S_{t+r}$ in solution that corresponds to the formation of a "rigid" complex[7] (where there are no residual motions of the individual components), in general agreement with the conclusions of Finkelstein and Janin.[5] Either larger amplitude motions in the crystal at the T_m ($\Delta G = 0$) are required to account for the smaller entropy changes observed in the fusion process and/or molecules in the melt remain partially ordered by the forces that hold them together in the crystal. Evidence to support this latter factor lies in the observation that enthalpies of evaporation are frequently much larger (in the range 2–12 times greater)[17] than the enthalpies of melting. In aqueous solution, molecules that are complexed become completely separated upon dissociation by the presence of solvent molecules. In this regard, the fusion process probably does not represent a good model for entropy changes in molecular recognition processes that take place in aqueous solution.

Table I. Enthalpies and Entropies of Fusion for *n*-Alkanes and Their Cyclic Analogues

alkane	T_m (K)	ΔH_{fusion} (kJ mol^{-1})	$T\Delta S_{fusion}$ (kJ mol^{-1}, 300 K)
n-pentane	144	8.4	17.5
cyclopentane	180	0.6	1.0
n-hexane	178	13.1	22.1
cyclohexane	267	2.6	2.8
n-heptane	183	14.2	23.3
cycloheptane	265	1.8	2.0
n-octane	216	20.6	28.6
cyclooctane	258	2.4	2.5

The enthalpies and entropies of fusion of molecules with increasing globular symmetry indicate the large amounts of disorder present at lattice sites at temperatures below the melting point. Crystals, characterized by low entropies of fusion (<6 kJ mol^{-1}, $T\Delta S$ at 300K), often formed from molecules of cubic or hexagonal symmetry, appear to assume the same number of distinguishable orientations in both the liquid and the so-called "plastic" crystal.[18,21] Data for the linear and cyclic hydrocarbons C_5, C_6, C_7, and C_8 (shown in Table I) illustrate the point. The cycloalkanes are characterized by low enthalpies and entropies of fusion, the latter in the range 1 to 2.8 kJ mol^{-1} (TΔS), but have melting temperatures significantly higher than the linear chain analogues. The higher T_m values reflect the fact that there is no great entropic advantage associated with melting; molecular disorder in the crystal, just below the melting temperature, would appear to be comparable to that of a freely translating, rotating, and conformationally flexible molecule in the liquid. Since the cycloalkanes have essentially no free internal bond rotations, their low entropies of fusion, $T\Delta S_{fusion} \approx 0$, lead us to the conclusion that $T\Delta S^f_{t+r}$ is also close to zero. The notion of order–disorder transitions below the melting temperature of the crystal is supported by the observation of NMR line width changes in the solid at temperatures well below the melting point.[22] Cyclohexane, for example, melts at 6 °C, but a phase transition is detected at −87 °C where a line width narrowing from 26 to 6 Hz is observed.[22]

Sublimation as an Alternative Model for Complex Dissociation. The thermodynamics of sublimation present an alternative model for complex dissociation; the solid is again analogous to a complex, while the gas phase approximates to an ideal solution where intermolecular interactions are negligible (in contrast to the molecular order that appears to persist in the melt). We present an expression similar to eq 2 to describe the entropy of sublimation

$$T\Delta S_{sub} = T\Delta S^s_{t+r} + T\Delta S^s_r \quad (9)$$

where superscript s represents entropy changes for the process of sublimation. We note that changes in the frequency of internal bond stretching and bending modes are small for gas-phase to condensed-phase transitions[5,23] and do not contribute significantly to the entropy of sublimation.

The entropy of sublimation[18] of a large number of compounds (free of internal rotors) is also found to be significantly smaller than experimental or calculated gas-phase entropies of translation and rotation,[5] again highlighting the importance of motions in the crystal lattice which can guide the study of weak complexes. Considering urea as an example, the experimental value of $T\Delta S_{sub}$ at 300 K is 43.5 kJ mol^{-1}, only about half of the change in $T\Delta S_{t+r}$ of 84 kJ mol^{-1}, calculated from eqs 4 and 5, and assuming a

(21) Timmermans, J. *J. Phys. Chem. Solids* **1961**, *18*, 1.
(22) Aston, J. G. Plastic Crystals. In *Physics and Chemistry of the Organic Solid State*; Fox, D., Labes, M. M., Weissberger, A.; Interscience Publishers, Wiley: New York, 1963; Chapter 9, pp 543–582.
(23) Herzberg, G. *Molecular spectra and molecular structure*; Van Nostrand: Princeton, NJ, 1945.

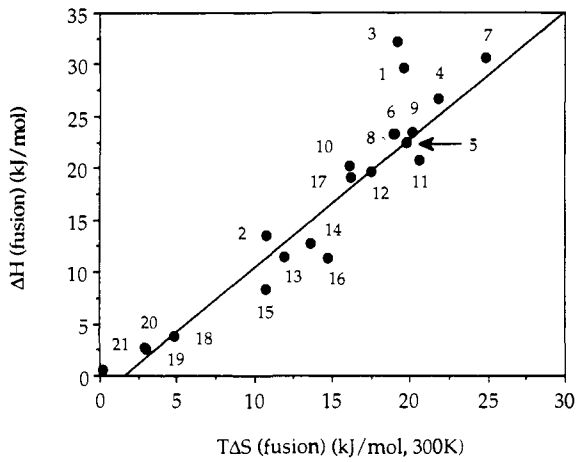


Figure 4. Enthalpy/entropy compensation in the fusion of 21 organic compounds (molecular mass up to a few hundred daltons). Plot of enthalpy of fusion versus entropy of fusion (at 300 K). Data are fitted to the equation $y = -2.2 + 1.3x$ with $R = 0.89$. The compounds considered are as follows: (1) 1,1-dimethylurea, (2) 1,3-dimethylurea, (3) 2,3,5-triiodobenzoic acid, (4) α,α'-dibromo-*o*-xylene, (5) 2,2',4',5-tetrachlorobiphenyl, (6) 2,7-dimethylnaphthalene, (7) 1,2'-dinaphthylmethane, (8) α-naphthol, (9) 2,5-dimethylphenol, (10) *o*-toluic acid, (11) 2-nitro-5-methylphenol, (12) 2,6-dinitrophenol, (13) acetic acid, (14) formic acid, (15) pyridine, (16) fluorobenzene, (17) naphthalene, (18) 1,2,4-trimethylbenzene, (19) tetrachloromethane, (20) cyclohexane, and (21) cyclopentane.

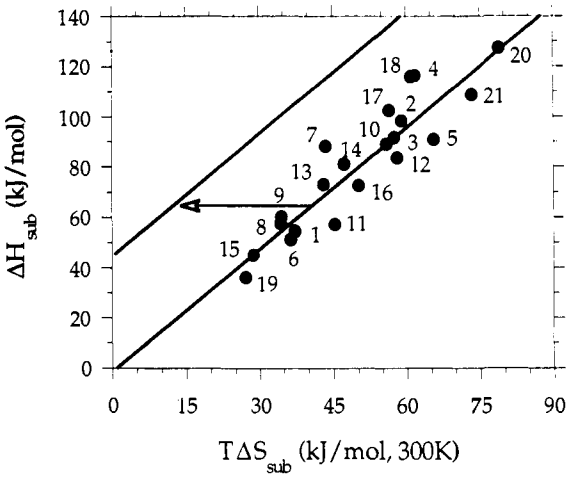


Figure 5. Enthalpy/entropy compensation in the sublimation of 21 organic compounds (molecular mass up to a few hundred daltons). Plot of enthalpy of sublimation versus entropy of sublimation (at 300 K). Data are fitted to the equation $y = -2.0 + 1.6x$ with $R = 0.92$. The compounds considered are as follows: (1) tetrabromomethane, (2) oxalic acid, (3) benzoic acid, (4) 4-hydroxybenzoic acid, (5) 4-chloroaniline, (6) quinuclidine, (7) urea, (8) acetamide, (9) 1,4-dibromobenzene, (10) 1-bromo-4-nitrobenzene, (11) 1,4-dichlorobenzene, (12) 1-chloro-4-nitrobenzene, (13) biphenyl, (14) hexamethylbenzene, (15) benzene, (16) naphthalene, (17) anthracene, (18) benzanthrone, (19) dicyanodiethyne, (20) 2-pyrrolecarboxylic acid, and (21) 2-furoic acid. The line displaced by $T\Delta S = -29$ kJ mol^{-1} corresponds to the Trouton correction for dissociation in solution (see text).

completely "rigid" crystal environment. The difference, some 40 kJ mol^{-1},[8] attributable to the entropy of motions of urea molecules in the crystal is in part a measure of the thermodynamically favorable residual motion which amide–amide hydrogen bonds allow. More generally, entropies of sublimation appear to account for only 40–70% of the gas-phase entropies (estimated for the 20 compounds considered in Figure 5, for which entropies of sublimation are available[18]).

Enthalpy/Entropy Compensation. The phenomenon of "enthalpy/entropy compensation" is well documented[3,13] but sometimes poorly interpreted in terms of a satisfactory physical model. It has been suggested that compensation effects are a consequence of some unique property of solvation;[13] however, the phenomenon is too widely observed to be closetted as a property only of solutions. In Figure 4, we present a plot of ΔH_{fusion} versus $T\Delta S_{fusion}$ (at 300 K) for some 21 organic compounds.[24] Among these compounds, larger enthalpies of fusion are generally associated with more polar molecules, identifying a relationship between the strength of electrostatic interactions in the crystal and the magnitude of the enthalpy of crystallization. The price to be paid for a large negative enthalpy of crystallization is a large unfavorable entropy, corresponding to a high degree of difference in order between the crystal and the melt. Since the compounds considered are largely free of internal rotors, eq 1 can be approximated to

$$T\Delta S_{fusion} \approx T\Delta S^{f}_{t+r} \qquad (10)$$

However, the plot in Figure 4 shows that an adverse entropy change, corresponding to complete immobilization in the crystal, is not achieved even in the tightest available crystalline complex (i.e., very large negative enthalpy of crystallization). This indicates the importance of the favorable entropy of residual motions, and possibly also some residual order in the melt. Where electrostatic interactions are strongest (more negative enthalpy), the favorable entropy of disorder and intermolecular motions appear to be smallest, and presumably this generalization applies to molecular recognition complexes which are important in biology.

The same relationship between enthalpy and entropy is applicable to the process of sublimation; ΔH_{sub} is plotted against $T\Delta S_{sub}$ (at 300 K) in Figure 5 for a set of compounds[18] satisfying the same selection criteria as fusion (largely free of internal rotations). As anticipated, larger enthalpies of sublimation are found for the more polar molecules. Since the molecules considered are also of similar mass and shape, which we assume not to be ordered in the gas phase due to molecular associations, $T\Delta S_{sub}$ also provides an approximate measure of $T\Delta S^{s}_{t+r}$ (for the solid to gas transition; sublimation equivalent of eq 10).

The compensatory effects of entropies on enthalpies of sublimation are illustrated by considering a number of specific examples. While the entropy of sublimation of 4-chloroaniline is ca. 75% of the calculated gas-phase entropy, ΔS_{sub} for 1,4-dichlorobenzene is only ca. 50% of the gas-phase value. The small difference in relative molecular mass (RMM) between the two compounds amounts to a difference in the calculated gas-phase entropy of translation and rotation ($T\Delta S$ at 300 K) of <2 kJ mol^{-1}. The difference in $T\Delta S_{sub}$ values for the two compounds of ca. 20 kJ mol^{-1} is largely accounted for by the difference in the entropy of residual motions in the crystal, which is larger for 1,4-dichlorobenzene than for the more polar 4-chloroaniline. This entropic difference is more than compensated by a large difference in ΔH_{sub} for the two compounds of 33.6 kJ mol^{-1}, reflecting much stronger intermolecular interactions in the crystal of the more polar 4-chloroaniline. When an amino group of urea is replaced by a methyl group in acetamide, the hydrogen bonding potential in the crystal is reduced. While the entropy of sublimation of urea is 43.5 kJ mol^{-1} ($T\Delta S$ at 300 K) that of acetamide is only 34.5 kJ mol^{-1}, corresponding to ca. 50% and 40%, respectively, of the gas-phase entropies, and a difference in $T\Delta S_{sub}$ of 9 kJ mol^{-1}. Urea has an enthalpy of sublimation of 88.2 kJ mol^{-1}, and acetamide 57.5 kJ mol^{-1}. The difference in enthalpy of 30.7 kJ mol^{-1} more than offsets the entropy difference. The results show that tight-binding (more negative enthalpy) in all cases is capable of offsetting the higher entropic cost of forming a tight complex.

Entropy Changes for Molecular Associations in Solution. Fusion and sublimation provide instructive models for features of complex dissociation, but for processes of biological interest it is the change in entropy in solution that might provide insight into molecular recognition phenomena. The possibility of partial ordering of molecules in the melt and examination of the transition at $\Delta G = 0$ suggest that entropies of fusion are probably less than those

(24) Acree, W. E., Jr. Enthalpy of fusion of some organic compounds. In *CRC Handbook of Chemistry and Physics*, 72nd ed.; Lide, D. R., Ed.; CRC Press Inc.: Boston, MA, 1991.

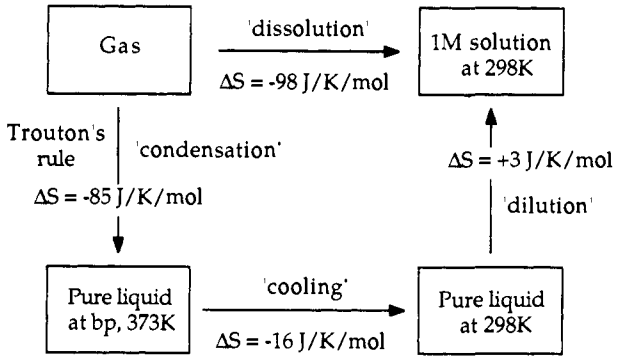


Figure 6. Thermodynamic cycle for estimating the change in entropy for converting 1 mol of gas (under standard conditions) to a 1 M solution at 298 K.

for the dissociation of complexes in solution; the entropies of gases are significantly larger than those of liquids, making entropies of sublimation an overestimate of the entropy changes for dissociation. However, the sublimation model for complex dissociation has the advantage that molecules are likely to be completely dissociated in the gas phase, as they might be in dilute solution. The observation that the entropies of condensation of a large number of liquids are found to be approximately constant at −87 J K^{-1} mol^{-1} (Trouton's rule) and independent of boiling point[25] enables us to correct the entropy of sublimation data to a value more representative of complex dissociation in solution. Two additional small corrections are required; Trouton's rule considers condensation to a "pure liquid" at its boiling point, which must be corrected for cooling to 298 K and dilution to 1 M solution. The entropy change on cooling from temperature T_2 to T_1 is estimated from the expression

$$\Delta S = -C_p \ln (T_2/T_1) \quad (11)$$

where C_p is the heat capacity at constant pressure. If we assume these parameters to be similar to those for water, i.e. cooling from 373 to 298 K and C_p = 75 J K^{-1} mol^{-1}, then a cooling correction of ca. 5 kJ mol^{-1} ($T\Delta S$ at 298 K) is estimated. The entropy of mixing of two ideal liquids A and B is given by

$$\Delta S_{mix} = R[X_A \ln (X_A) + X_B \ln (X_B)] \quad (12)$$

where X_A and X_B are the mole fractions of A and B, respectively.[26] Dilution from a 10 M "pure liquid" to a 1 M solution, for example, results in a small entropy of mixing of only 3 J K^{-1} mol^{-1}. The entropy changes in the thermodynamic cycle from a gas to a 1 M solution at 298 K are illustrated in Figure 6 and amount to a "modified" Trouton's rule correction of −29 kJ mol^{-1} ($T\Delta S$ at 298 K). Experimental data on the entropies of dissolution of gases in aqueous solutions under standard conditions[20] are found to give reasonably constant values of −29 kJ mol^{-1} ($T\Delta S$), in excellent agreement with the Trouton's rule estimate. A larger entropy change for methylamine of −36 kJ mol^{-1}, for example, suggests that ordering of water may make a contribution to solvation entropies, but even including unusual cases, values are typically in the range −24 to −36 kJ mol^{-1}.

Thus, a correction of −29 kJ mol^{-1} ($T\Delta S$) to the entropies of sublimation of the compounds in Figure 5 gives a guide to the net entropic cost of motions lost in complex formation. The conclusion to be drawn is that molecules which form only weak interactions in the crystal (small enthalpies of sublimation) are associated with correspondingly small (in some cases close to zero) entropy changes for complex formation in solution. In the case of the more polar molecules in Figure 5, the predicted entropic cost of binding is significantly larger, ca. −45 kJ mol^{-1} (i.e., $T\Delta S_{sub}$ − Trouton's correction), and much closer to the accepted range of values for the reduction in entropy of 50–60 kJ mol^{-1} derived by Page and Jencks[2] for covalent bond formation. We reiterate that bimolecular associations with very little exothermicity may have remarkably small adverse entropies. The benefit of translational and rotational entropy which remains in the complex, plus the entropic advantage of residual motions and new low-frequency vibrations, may in some cases almost balance the formal loss of $T\Delta S_{t+r}$ associated with the formation of a rigid complex. In the dimerization of 2-pyridone in chloroform,[27] the formation of a rigid dimer would cost (before considering the advantage of residual motions and soft vibrations) ca. 10^{10} M^{-1} in binding constant due to loss of rotational and translational entropy.[2] The experimental adverse entropy at 298 K is found to be −13.5 kJ mol^{-1} ($T\Delta S$), opposing binding by only $10^{2.3}$ M^{-1}. Thus, the advantage of residual motions in the complex (which includes the entropy of the two NH···O=C low-frequency vibrations) promotes binding by $10^{7.7}$ M^{-1}. When this entropic benefit of $10^{7.7}$ M^{-1} is added to the favorable enthalpy term for the formation of two hydrogen bonds of $10^{4.4}$ M^{-1} (ca. −25 kJ mol^{-1}), a large favorable overall free energy change for dimerization of 10^2 M^{-1} is the result.

In aqueous solutions, the entropically beneficial effects of hydrogen bond formation in bimolecular associations may be even larger than in nonpolar solvents. We are led to this conclusion because such hydrogen bonds in water have little exothermicity,[28,29] which the effect of enthalpy/entropy compensation permits us to associate with more residual motion in the complex. The weaker (alleged hydrogen bonded) dimerization of cyclic lactams in water (ca. 10^{-1} M^{-1}) relative to carbon tetrachloride (ca. 10^2 M^{-1}) has been widely accepted and cited as evidence for the weakness of this hydrogen bond in water.[30]

We had recently concluded,[8,11] using data for the association of urea, cyclic lactams, and diketopiperazine, that amide–amide hydrogen bonds in aqueous solution can have binding energies in the range −13 to −24 kJ mol^{-1}, which equate with binding selectivities of $10^{2.3}$ to $10^{4.2}$ M^{-1}. Such binding energies are greater than the values generally attributed to hydrogen bonds;[28-33] the amide–amide hydrogen bond and other neutral hydrogen bonds are generalized to have binding energies of −2 to −7.5 kJ mol^{-1} (binding selectivities in the range 2–20).[28,29,34] The major part of the difference between these two sets of binding selectivities lies in the treatment of residual motions in deriving these values. Large free energy values arise if the hydrogen bond is credited with the entropic advantage of residual motions, and small values if it is not. After correction for such residual motions, the values are compatible within the uncertainties which can be ascribed to experimental error, as discussed in the following paper.[9]

Ligand–Receptor Interactions in Aqueous Solution. Fundamental differences in the thermodynamics of molecular interactions between agonists and antagonists binding to a common receptor point to the importance of enthalpy/entropy compensations in their mechanisms of action. In important papers, two investigations have highlighted the binding of agonists and antagonists to β-adrenergic receptors.[14,15] Agonists (frequently evolved under the influence of natural selection), characterized by their ability to elicit a conformation response in a receptor upon binding, are found to bind with large negative enthalpies. Antagonists, which act by blocking the same receptor site, are generally observed to bind with much less negative (in some cases slightly positive) enthalpies and much less negative (generally positive) entropies than agonists. In Figure 7, we plot ΔH versus $T\Delta S$ (at 300 K) for the binding of agonists (●), partial agonists (○), and antagonists (▲) binding

(25) Everett, D. H. *J. Chem. Soc.* 1960, 2566–2573.
(26) Atkins, P. W. *Physical Chemistry*; Oxford University Press: London, 1982; pp 169–170.
(27) Ducharme, Y.; Wuest, J. D. *J. Org. Chem.* **1988**, *53*, 5787–5789. Hammes, G. G.; Park, A. C. *J. Am. Chem. Soc.* **1969**, *91*, 956–961.
(28) Fersht, A. R.; Shi, J.-P.; Knill-Jones, J.; Lowe, D. M.; Wilkinson, A. J.; Blow, D. M.; Brick, P.; Carter, P.; Waye, M. M. Y.; Winter, G. *Nature* **1985**, *314*, 235–238.
(29) (a) Fersht, A. R. *Trends Biochem. Sci.* **1987**, *12*, 301–304. (b) Fersht, A. R. *Enzyme Structure and Function*; W. H. Freeman: New York, 1985.
(30) Baldwin, R. L. *Trends Biochem. Sci.* **1989**, *14*, 291–294.
(31) Dill, K. A. *Biochemistry* **1990**, *29*, 7133–7155.
(32) Pace, C. N.; Heinemann, U.; Hahn, U.; Saenger, W. *Angew. Chem.* **1991**, *30*, 343–360.
(33) Murphy, K. P.; Gill, S. J. *Thermochim. Acta* **1990**, *172*, 11–20.
(34) Shirley, B. A.; Stanssens, P.; Pace, C. N. *Biochemistry* **1992**, *31*, 725–732.

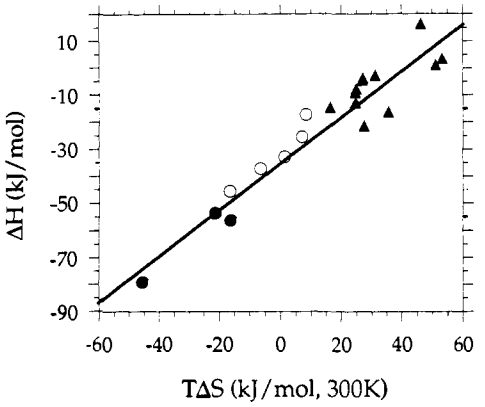


Figure 7. Enthalpy/entropy compensation in the binding of agonists, partial agonists, and antagonists to β-adrenergic receptors (data from refs 14 and 15). Enthalpy of binding (ΔH) plotted against entropy of binding ($T\Delta S$) at 300 K. Data are fitted to the equation $y = -34.1 + 0.87x$ with $R = 0.95$. Key: (●) agonists, (○) partial agonists, (▲) antagonists.

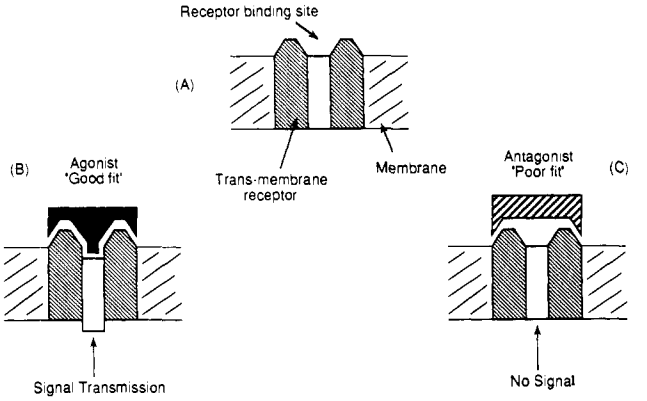


Figure 8. Schematic representation of complementarity/response effects of agonist and antagonist binding to a common receptor site: (A) unmodified membrane-bound receptor, (B) agonist bound to the active form of the receptor (good complementarity elicits a conformational response that is transmitted across the membrane), (C) antagonist bound to the receptor ground state (poor complementarity elicits no conformational response, but blocks the receptor site).

to a β-adrenergic receptor.[14,15] We do not single out any one of the three classes of ligand, but present the data in a single plot to highlight the remarkable correlation between enthalpy and entropy of binding. While this observation is not new,[14,15] we have reconsidered the current interpretation of this phenomenon in the light of our earlier investigations of enthalpy/entropy compensations in the processes of fusion and sublimation.

Bimolecular associations in aqueous solutions are opposed by the loss of translational and rotational entropy. However, as emphasized earlier in this paper, an association of small or negligible exothermicity can lead to a small or negligible loss of translational and rotational entropy. In this sense, it is more sensible not to treat very weakly exothermic associations (having small electrostatic barriers to dissociation) as those which lose a large amount of translational and rotational entropy but then almost offset this with a large favorable entropy of new vibrations; rather, it is better to note that the small entropic cost is simply a consequence of weak electrostatic binding. In aqueous solution, there are several additional sources of positive entropy (favorable to binding) that are associated with the properties of water and which do not have a counterpart in the processes of fusion and sublimation. The first of these is the hydrophobic interaction and the second is the possibility that there is randomization of water molecules associated with the formation of at least some hydrogen bonds that may be entropically favorable (perhaps most important for interactions involving charged entities).[7,8,11,29] The large swing from small negative entropy of binding of agonists to a large positive entropy of binding for antagonists is probably to an important extent a consequence of the enthalpy/entropy compensations alluded to above.

Where enthalpies of binding are large and negative (agonists), we conclude that there are strong electrostatic interactions between the *bound* conformations of the agonist and receptor. As a consequence of these strong electrostatic interactions, motion of the bound ligand will be much reduced relative to its free state, with a consequently relatively large loss in translational and rotational entropy. Note that since agonist binding is characterized by signal transmission, a conformational change is likely to occur at the receptor when it binds ligand. Since the receptor undergoes a conformational change from one that is an energy minimum when free to one that is not, at the demand of the ligand, then clearly the ligand must make relatively strong electrostatic interactions to the *bound* conformation of the receptor.[35] That these interactions are still strong even after the enthalpic cost of the conformational change in the receptor has been paid is indicated by the large and negative enthalpies of binding of agonists. Strong electrostatic interactions with ligand are necessary for conformational change at the receptor surface and for conformational transmission within it. The cost of such exothermic binding is a relatively large loss of translational and rotational entropy of the ligand.

Antagonists on the other hand do not fulfill the requirements of good complementarity to the receptor conformation induced by agonists, as evidenced by the criterion that their enthalpies of binding are small. The loss in translational and rotational entropy of the antagonists is therefore less than that of the agonists. It is therefore possible to have similar binding constants for agonists and antagonists; but the looser binding of the antagonists does not permit transmission of a conformational change, and thus there is no physiological response in this case. It is noteworthy that a bimolecular association can be associated with a positive entropy change if the loss in translational and rotational entropy, and of any internal rotations, is more than offset by the gain in entropy associated with any hydrophobic effect [see points for antagonists (▲) in Figure 7]. These contrasts between agonist and antagonist binding to a common receptor are represented schematically in Figure 8.

A more recent interpretation[14] of the thermodynamic data differs in the origin of positive entropy changes on binding. Our model is in contrast to the proposal that a positive entropy change for antagonist binding is a consequence of a transition from some membrane bound intermediate state to a loosely bound state in the binding cavity. Miklavc et al.[14] appear to model the binding process only in terms of a transition from a membrane-bound state of low entropy to one in which the ligand is bound in a high-entropy state in the binding cavity. The correct interpretation must consider the entropy changes associated with transferring the ligand from solution to its final bound state. The positive entropy changes associated with antagonist binding may find their origin in part in some combination of release of ordered water molecules from polar groups and from the surface of hydrocarbon that becomes buried from solvent. Additionally, the receptor may possess more residual motion when binding antagonist than when binding agonist, and the antagonist structures themselves are anticipated to be less ordered when bound than are the agonist structures. The entropy-driven binding mechanism for the antagonists becomes evident when the ligand is weakly bound (small negative enthalpy) and entropy changes due to loss of translational and rotational freedom are to a larger degree offset by the entropy of motions in the complex.

Enthalpy/entropy compensations in host–guest interactions have been clearly demonstrated for the binding of tetrasaccharides to lectins,[13a] where it is proposed that such compensations are largely a manifestation of a unique property of water. The rationalization of this phenomenon in the examples described above lead us to an alternative view, and one already suggested in part by Carver

(35) Jencks, W. P. *On the Economics of Binding Energies*; Proc. of the XVIIIth Solvay Conference on Chemistry, Nov. 1983, Brussels, Belgium; van Binst, G., Ed.; Springer-Verlag: Berlin; 1986; p 75.

et al.[36] The experimental data indicate that deoxygenation (dehydroxylation) of tetrasaccharides results in both a less negative enthalpy and a less negative entropy of binding. In line with the hypothesis presented earlier, we propose that the entropy of binding becomes more negative (unfavorable) as the extent of the oligosaccharide interaction with the protein interface increases (more negative enthalpy of interaction). Lemieux et al.[13a] propose that deoxygenation results in a less negative enthalpy of binding because a substrate hydroxyl group in the "free" ligand is surrounded by "high-energy" water which contributes a favorable enthalpy of binding when such water molecules are released to bind more strongly to the bulk solvent. This proposal is inconsistent with the conclusion that neutral-neutral hydrogen bonds in aqueous solution have only small or negligible exothermicities.[29a]

(36) Carver, J. P.; Michnick, S. W.; Imberty, A.; Gimming, D. A. Oligosaccharide-protein interactions: a three-dimensional view. In *Carbohydrate Recognition in Cellular Function*; Ciba Foundation Symposium; Wiley: Chichester, 1989; Vol. 145, pp 6-26.

Conclusions

The thermodynamics of phase transitions (melting and sublimation) undergone by organic crystals have been used to suggest the approximate entropic cost of rotor restrictions, and the loss in translational and rotational motions, appropriate to the formation of weakly bound complexes in solution. The data indicate that the loss in translational and rotational entropy increases gradually from very small to limiting values as the endothermicity of the dissociation increases. Some β-adrenergic agonists bind with relatively large exothermicities; this presumably permits the formation of a relatively high ordered agonist/receptor complex, suitable for precise conformational change and hence for signalling at a distance.

Acknowledgment. We thank the SERC, the Upjohn Company (Kalamazoo), and Roussel Uclaf for financial support.

Partitioning of Free Energy Contributions in the Estimation of Binding Constants: Residual Motions and Consequences for Amide-Amide Hydrogen Bond Strengths

Mark S. Searle, Dudley H. Williams,* and Ute Gerhard

Contribution from the Cambridge Centre for Molecular Recognition, University Chemical Laboratory, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK.
Received June 15, 1992

Abstract: Any bimolecular association is entropically unfavorable because of degrees of freedom of translation and rotation lost when two molecules come together to form a complex. For a ligand of molecular weight 200, the formation of a "rigid" dimer (one in which there is no residual relative motion of the associating components **A** and **B** in the complex **A·B**) opposes binding by ca. 10^{-9} to 10^{-10} M^{-1} in binding constant. If relative motions (including new soft vibrations) in the complex are then credited to the functional group interactions then the amide-amide hydrogen bonds, for example, those involved in the reported formation of lactam dimers in solution, are concluded to promote dimerization by ca. 10^4 per hydrogen bond (Doig, A. J.; Williams, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 338). An alternative approach is to regard residual relative motions remaining in the complex as constituting translational and rotational entropy of **A** and **B** that was not lost. In this paper and in the preceding paper we have attempted to quantitate the contribution of residual motions in weakly bound complexes from literature data on the fusion, sublimation, and dissolution of model compounds. If the entropic advantage of the residual motions is removed as entropy that is not lost in the bimolecular association, then free energies for amide-amide hydrogen bond formation are obtained that are not significantly different from the conventional view of these bonds of between −2 and −8 kJ mol^{-1}. The same conclusion is reached in ligand extension studies for the binding of peptide cell wall analogues to the antibiotics vancomycin and ristocetin A if credit for residual motions is removed, and allowance is made for a larger hydrophobic effect than originally envisioned (Williams, D. H., et al. *J. Am. Chem. Soc.* **1991**, *113*, 7020).

Introduction

In several recent publications,[2-4] we have considered an approach to the factorization of the free energy of binding for molecular associations in aqueous solution, by partitioning free energy contributions into four principal terms. Our analysis is based upon the pioneering work of Jencks[5] and Page and Jencks.[6] A similar factorization has previously been used by Andrews et al.,[7] and the relevance and physical basis of the factors involved have been summarized by Fersht.[8] The consideration of only four terms is justified only if the ligand and receptor show good van der Waals complementarity, and if the conformations of the bound components correspond closely to conformational energy minima in the separated states.[2,3] These terms are considered as

(1) Abbreviations: ΔG, ΔH, and ΔS, change in Gibb's free energy, enthalpy and entropy; K, binding constant; T, temperature (K); T_m, melting temperature (K); ΔG_{t+r} and ΔS_{t+r}, change in free energy and entropy of translation and rotation; ΔG_r and ΔS_r, change in free energy and entropy of internal rotations; ΔG_p, change in free energy for polar group interactions; ΔG_h, change in free energy due to the hydrophobic effect; ΔG_{conf}, change in free energy due to conformational strain; ΔG_{vdW}, change in free energy due to van der Waals interactions; ΔA_{np}, change in nonpolar surface area (Å^2).

(2) Williams, D. H.; Cox, J. P. L.; Doig, A. J.; Gardner, M.; Gerhard, U.; Kaye, P. T.; Lal, A. R.; Nicholls, I. A.; Salter, C. J.; Mitchell, R. C. *J. Am. Chem. Soc.* **1991**, *113*, 7020-7030.

(3) Williams, D. H. *Aldrichim. Acta* **1991**, *24*, 71-80.

(4) Doig, A. J.; Williams, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 338-347.

(5) Jencks, W. P. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4046-4050.

(6) Page, M. I.; Jencks, W. P. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1678-1683.

(7) Andrews, P. R.; Craik, D. J.; Martin, J. L. *J. Med. Chem.* **1984**, *27*, 1648-1657.

(8) Fersht, A. R. *Enzyme Structure and Mechanism*, 2nd ed.; W. H. Freeman: New York, 1985.

0002-7863/92/1514-10697$03.00/0 © 1992 American Chemical Society