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Thermodynamics of Supramolecular Structure Formation in Water

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1. Introduction

Hydrophobic interactions play important roles in various aspects in physics, chemistry, and biology. In particular, binding of biological molecules to proteins, polynucleotides, and cell membranes is driven by hydrophobic interactions as well as other forces such as London's dispersive interactions, van der Waals interactions, electrostatic interactions, charge-transfer interactions and hydrogen bonding (Ben-Naim & Marcus, 1984; Schneider & Yatsimirsky, 2000; Southall et al., 2002; Mayer et al., 2003; Houk et al., 2003). Hydrophobic interactions are also essential in formation of self-assemblies with a nanometer size, and construction of supramolecular structures on the interface between two different phases (Chandler, 2005). In this chapter we discuss thermodynamics of binding of a non-polar molecule to a receptor molecule/polymer, with particular attention to the hydrophobic/hydrophilic environment of the binding region as a critical factor to direct the mechanism of hydrophobic interactions. In particular, we show that enthalpy-entropy compensation would work only weakly or even non-compensation was observed when the hydrophobic interactions are extracted from various binding forces.

Origin of the hydrophobic interactions was attributed to the formation of hydrogen bonds of water molecules on the surface of non-polar molecules. To understand the thermodynamics of binding of a molecule to a biopolymer, however, other dynamics such as conformational changes in polymer and direct intermolecular interactions between the molecule and the polymer should be taken into consideration in addition to the dynamics of hydrogen bonding formation/destruction between water molecules.

The association constants of ligands to proteins for 160 protein-ligand pairs were distributed with an average of $10^{9.3} \text{ M}^{-1}$ (Kunz et al., 1999). Thermodynamic studies of binding of simple molecules to synthetic receptors such as cyclodextrins and cyclophanes have been carried out to shed light on the molecular picture of hydrophobic interactions. The association constants of various ligands to cyclodextrins were smaller than those of ligands to proteins and the average was $10^{2.4} \text{ M}^{-1}$ (Rekharsky & Inoue, 1998). Much tighter binding to proteins implies that proteins have been highly developed as a receptor to have both high affinity to the ligand and high selectivity. Understanding of the mechanisms of binding is needed and much work has been performed to clarify the molecular mechanism of host-guest interactions.

According to the classical theory of hydrophobic interactions (Frank & Evans, 1945), the driving force of the attractive interaction should be entropic gain due to increased freedoms

of water molecules. It is puzzling, however, that hydrophobic effects are enthalpy-driven in some cases (Ross & Subramanian, 1981; Gelb et al., 1981; Smithrud et al., 1991; Spencer et al., 1995; Arena et al., 2000) and entropy-driven in others (Hooley et al., 2007; Iwamoto et al., 2007; Whitesides & Krishnamurthy, 2005; Sgarlata et al., 2010). Jencks proposed that hydrophobic interactions can be classified into two categories, (1) classical hydrophobic interactions with an entropy term as a driving force and (2) non-classical hydrophobic interactions with an enthalpy term as a driving force (Jencks, 1969). According to his proposal, the classical hydrophobic interactions operate for a non-polar host-guest complex, while the non-classical hydrophobic interactions operate for a relatively polar host-guest complex.

We have made systematic studies on thermodynamics of binding of an alkyl group to synthetic receptors consisting of a hydrophobic porphyrin framework and poly(ethylene oxide) auxiliary groups of varying degrees of polymerization (Mizutani et al., 2003; Iwamoto et al., 2007; Matsumoto et al., 2009). We revealed that the driving force of binding of an alkyl group changed from enthalpy to entropy as the fraction of poly(ethylene oxide) moieties increased. We proposed that water accessibility to the host-guest interacting region affects the driving force of hydrophobic interactions: the binding is entropy-driven if the binding pocket is sequestered from water, while it is enthalpy-driven if the binding pocket is open to water. Thermodynamic parameters of binding of alkyl groups to proteins also showed very diverse compensation temperatures, indicating that hydrophobic interactions are characterized by the non-compensation effects of enthalpy and entropy. The findings are important to interpret the thermodynamic data of binding of biological interest, and also to rational design of drugs based on protein structures (Talhout et al., 2003; Malham et al., 2005).

2. Hydrophobic interactions

2.1 Hydrophobic interactions

Water is a solvent used in many chemical and biological reactions, and the unique feature of water is dynamic behavior of formation of hydrogen bonding between water molecules. Formation and destruction of hydrogen bonds occur very rapidly and frequently in liquid water, so that the dynamics of hydrogen bonding affects the reactions and equilibria in water to a significant way. One of such effects in water is called hydrophobic effects or hydrophobic interactions, and they are driving forces of association of non-polar molecules in water. Hydrophobic interactions operate in diverse fields such as binding of organic molecules to proteins, a folding process of proteins, double helix formation of polynucleotides, action of detergents on the interfaces, and formation of cell membranes.

In particular, hydrophobic interactions play an important role in affinity and selectivity of binding of an organic molecule to protein, and understanding of the molecular mechanism of hydrophobic interactions is quite important for the rational design of drugs, for instance. However, the hydrophobic interactions are statistical effects of the solvent, and the protein molecule has many freedoms of motion. These two factors make the molecular picture of binding mechanism of biomolecules through hydrophobic effects very complex.

2.2 Iceberg formation on non-polar molecules in water

Hydrophobic interactions originate from the solvation of non-polar molecules. Frank and Evans pointed out that, in a solution of noble gas or a non-polar molecule in water, the water molecules near the surface of non-polar molecule have greater „crystallinity“ (Frank & Evans, 1945). These non-polar molecules build a microscopic iceberg around them. Formation of

iceberg can explain the fact that low solubility of non-polar molecules in water originates from the unfavorable entropy. Nemethy and Scheraga calculated the statistics of water molecules which has four hydrogen bonds, three hydrogen bonds, two hydrogen bonds, and one hydrogen bond (Nemethy & Scheraga, 1962a, 1962b). The water molecule with four hydrogen bonds is most stable in terms of enthalpy (the potential energy), but is most unstable in terms of entropy since formation of multiple hydrogen bonds restricts the translational and rotational motion of the water molecule. In the opposite sense, the water molecule with one hydrogen bond is most unstable in terms of enthalpy, but is most stable in terms of entropy. The thermodynamic equilibrium will be established where the two competing terms, enthalpy and entropy, compensate to reach the minimum of the free energy.

2.3 Enthalpy and entropy changes upon transfer of alkane in water to alkane in the liquid state

Thermodynamic data of transfer of a series of alkanes from water to alkanes in the liquid state give us valuable insight into the mechanism of hydrophobic interactions. According to the iceberg structure formation theory near the surface of a non-polar molecule, thermodynamic parameters of the hydrophobic solvation or hydrophobic interactions should be proportional to the surface area in contact with water. Proportionality of the free energy and the surface area of non-polar moiety of the molecules, i.e., the solvent accessible surface area, has been confirmed for a number of thermodynamic processes such as solubilization of alkanes in water, partition of various molecules between water and organic solvent, protein unfolding, and protein-ligand binding (Hermann, 1972; Reynolds et al., 1974; Sharp et al., 1991a; 1991b; Richards, 1977; Böhm, 1994; Cohen & Connors, 1970; Harris et al., 1973).

Fig. 1. Plot of the chemical potential changes in transfer of alkanes from water to pure liquid alkane, $\Delta\mu^\circ$, against the carbon number of alkanes (ethane, propane, butane, pentane, and hexane). $\Delta\mu^\circ = \mu_{\text{HC}}^\circ - \mu_{\text{W}}^\circ$. The standard states are defined as follows: chemical potential of alkane in water: $\mu_{\text{W}} = \mu_{\text{W}}^\circ + RT \ln X_{\text{W}} + RT \ln f_{\text{W}}$, where X_{W} is mole fraction of alkanes, and f_{W} is the activity coefficient. μ_{HC} denotes chemical potential of alkane: $\mu_{\text{HC}} = \mu_{\text{HC}}^\circ + RT \ln X_{\text{HC}} + RT \ln f_{\text{HC}}$, where X_{HC} is mole fraction of alkanes, and f_{W} is the activity coefficient. Least squares line fit gave $\Delta\mu^\circ = (-4.03 \pm 0.03)n - (8.4 \pm 0.3)$, where n is the carbon number. Data were taken from Tanford (1973)

Fig. 2. Plot of the enthalpy change in transfer of alkanes (ethane, propane, butane, pentane, and hexane) from an aqueous solution to pure liquid alkane. Least squares line fit gave $\Delta H^\circ = (-2.6 \pm 0.3)n - (15 \pm 1)$, where n is the carbon number. Data were taken from Tanford (1973)

Fig. 3. Plot of the entropy change in transfer of alkanes (ethane, propane, butane, pentane, and hexane) from an aqueous solution to pure liquid alkane. Least squares line fit gave $\Delta S^\circ = (5.4 \pm 0.4)n - (76 \pm 2)$, where n is the carbon number. Data were taken from Tanford (1973)

First, we discuss the process of transfer of linear alkane ($\text{CH}_3(\text{CH}_2)_{n-2}\text{CH}_3$) in water to alkane in its liquid state. Figures 1-3 illustrate plots of free energy, enthalpy, and entropy changes in transfer of alkanes from water to pure alkane in the liquid phase against the carbon numbers of alkanes, n . All these thermodynamic parameters showed linear correlation with the carbon number. From the slopes of the lines we estimated the free energy change, the enthalpy change, and the entropy change per one CH_2 group as -4.0 kJ mol^{-1} , -2.6 kJ mol^{-1} , and $+5.4 \text{ J K}^{-1} \text{ mol}^{-1}$, respectively. The negative sign of the free energy change indicates that

the transfer of alkane from water to alkane is a favorable process, and that hydrophobic effects, or hydrophobic forces, are attractive interactions. It is interesting to note that both the enthalpy term and the entropy term contributed favorably to the free energy changes. The favorable contribution from the entropy term is explained by cleavage of hydrogen bonds of water upon transfer of alkanes, that is, dissolution of the iceberg structure. The large negative enthalpy change implies that attractive interactions between alkane molecules in the liquid state such as attractive van der Waals forces (London's dispersive forces) make a significant contribution to the overall thermodynamics of the process.

2.4 Enthalpy and entropy changes upon transfer of alkane in water to gaseous alkane

In several studies, hydrophobic interactions have been discussed based on the thermodynamic parameters in the transfer processes of alkane or noble gas in the gaseous state to those in water (Abraham, 1982; Blokzijl & Engberts, 1993). On the basis of the solubility of linear alkanes with carbon numbers 2-8, contribution of one methylene group to ΔG° , ΔH° and $T\Delta S^\circ$ of the transfer process are estimated to be $-0.75 \text{ kJ mol}^{-1}$, $+2.80 \text{ kJ mol}^{-1}$, and $+3.56 \text{ kJ mol}^{-1}$, respectively. The enthalpy term and the entropy term drive the equilibrium to the opposite directions and the free energy changes are dominated by the positive entropy term. When the thermodynamic parameters of the transfer of alkane in water to liquid alkane are compared with those to gaseous alkane, the enthalpic change is negative in the former case. It implies that the attractive van der Waals interactions between alkane molecules in the liquid state play an important role.

2.5 Molecular picture of hydrophobic interactions

These thermodynamic parameters lead to the picture of classical hydrophobic interactions: the association of non-polar molecules in water is driven by the extinction of hydrogen bonded water molecules on the non-polar surface, which should be entropically favorable process where the freedom of water molecules increases. The positive slope of the plot of the entropy changes vs. carbon number for the transfer of alkane from water to organic solvent (Figure 3) reflects the increased freedom of water molecules after non-polar molecules are removed from water. If only the destruction of the iceberg structures is considered, the process should be enthalpically unfavorable since some of the hydrogen bonds in the iceberg are broken. Figure 2 shows that the slope of the plot of the enthalpy changes vs. carbon number is, however, negative. Therefore, the attractive interactions between alkanes in the liquid state overcome the enthalpic cost to cleave the hydrogen bonds in the iceberg.

The interactions involved in the protein-substrate complex formation are composed of several forces such as hydrogen bonding, hydrophobic interactions, polar interactions, ionic interactions, and so on. Thus, diverse interactions should contribute to the thermodynamic parameters of binding of ligands to proteins.

We focus on the binding of alkyl groups to a synthetic receptors or proteins to clarify the molecular picture of hydrophobic interactions in detail.

3. Enthalpy – entropy compensation

Binding of guest to host is determined by the free energy changes of the equilibrium, and the free energy can be separated into two terms: the enthalpy term and the entropy term.

Physical meaning of the two terms is quite different, the enthalpy term originates from the potential energy of the system while the entropy term from the motional freedom of the system. Two terms, however, often counteract each other to ensure stability of the thermodynamic equilibrium. In a number of processes, the potential energy term tends to restrict the motional freedom of the system to lead to smaller entropy. The entropy term tends to disturb the system to a disordered state, and this would result in larger enthalpy. The balance of these two terms finds a thermodynamic equilibrium point, where the free energy becomes minimum.

Before discussing the mechanism of hydrophobic interactions, we summarize here enthalpy-entropy compensation found in a number of reactions and equilibria. Particularly, binding of various molecules to biopolymers or other receptor molecules is characterized by an enthalpy and entropy compensation (Lumry & Rajender, 1970; Gilli et al., 1994). For a series of host-guest pairs with one of these structures being systematically altered, the enthalpy changes and the entropy changes in binding are often linearly correlated. The plot of $\delta\Delta S^\circ$ vs. $\delta\Delta H^\circ$ gives a line with a positive slope $1/T_c$.

$$\delta\Delta S^\circ = (1/T_c)\delta\Delta H^\circ + \beta \quad (1)$$

T_c is called a compensation temperature. The positive slope indicates that the enthalpy-entropy compensation operates, while the negative slope indicates that the enthalpy term and the entropy term cooperate to result in large free energy changes. When the equilibrium is established at T_c , the free energy change can be given by equation (2).

$$\delta\Delta G^\circ = \delta\Delta H^\circ - T_c\delta\Delta S^\circ \quad (2)$$

Equation (2) is reduced to $\delta\Delta G^\circ = \beta$ by replacing $\delta\Delta S^\circ$ with equation (1). Therefore the entropy changes and the enthalpy changes due to the structural changes balance to lead to constant free energy changes at T_c . When T_c is close to room temperature, the strong compensation works, and the binding affinity remains the same even if the structures of ligands or drugs are altered. To find out a specific ligand to a target protein should be very difficult if this is the case.

Alternatively, in many literatures, $T\Delta S^\circ$ is plotted against ΔH° to show enthalpy-entropy compensation. In this plot, the slope of the line is T/T_c , where T is the temperature at which the binding data are collected.

In a number of instances of binding in water, T_c is close to room temperature. Lumry and Rajender suggested that various processes in water such as solvation of ions and nonelectrolytes, hydrolysis, and protein reactions follow the enthalpy-entropy compensation relationship with the compensation temperature in a relatively narrow range, from 250 to 315 K (Lumry & Rajender, 1970). Enthalpy-entropy compensation observed for organic reactions was reviewed by Leffler & Grunwald (Leffler & Grunwald, 1963). Dunitz suggested that enthalpy-entropy compensation is a general property of weak intermolecular interactions, and the enthalpy term and the entropy term should nearly balance out for a hydrogen bond at 300 K (Dunitz, 1995).

The two characteristic parameters, compensation temperature, T_c , and the entropy changes at zero enthalpy changes (β) as shown in equation (1) have been determined experimentally for binding of various ligands to receptors. Inoue and coworkers proposed that the

compensation temperature reflects the flexibility of the host-guest system: the high compensation temperature means rigid host-guest system where binding causes minimal structural changes (Rekharsky & Inoue, 1998). The ΔS° term reflects the solvation energy where large ΔS° means large changes in solvation during host-guest complexation.

It should be noted, however, that enthalpy-entropy compensation was not observed for the transfer of alkane from water to pure alkane as shown in Figures 1-3. Increasing the carbon number caused the negative free energy, negative enthalpy and positive entropy changes. Similar non-compensation was observed for the binding of an alkyl group to synthetic receptors (see below).

4. Perturbation approach to access hydrophobic interactions: Synthetic receptors

We can determine the free energy changes, the enthalpy changes and the entropy changes from the experiments either based on the equilibrium constants obtained with various physical or spectroscopic techniques or directly determine the heat flow using calorimetry. However these thermodynamic data contain several interactions and several motional freedom changes associated with different elementary processes. For instance, binding of substrates to proteins is driven by several interactions such as hydrophobic interactions, hydrogen bonding, electrostatic interactions (salt bridges between ions and dipolar interactions), van der Waals interactions, and coordinative interactions. These attractive or repulsive forces also cause freeze of some of the motional freedoms in host and guest. Translational and rotational motion of guest and internal bond rotation of host and guest would be frozen to some extent to result in the negative entropy changes. It is generally very difficult to extract hydrophobic interactions in a pure form from these data.

Large entropic cost to freeze the translational and rotational freedom of guest upon binding should be paid by a number of weak interactions (Knox, 1971; Tabushi et al., 1978; Mizutani et al., 1994). In this case, which pairwise interaction paid the entropic cost is not clear. To extract the interaction in a pure form, we could use a relatively strong interaction, which is strong enough to freeze the translational and rotational freedom of guest, and then additional weak interactions can be evaluated without interference from the enthalpy-entropy compensation, since the translational and rotational entropic costs have been already paid by the strong interactions. We designed synthetic receptors to evaluate hydrophobic interactions based on the strategy. The structures of the receptors are shown in Figure 4. The receptors have the zinc ion and the zinc acts as a Lewis acid to bind Lewis bases with an exothermic reaction (Kirksey et al., 1969; Mikros et al., 1988; Mizutani et al., 1999). The Lewis acid-Lewis base interactions between the zinc and pyridine are strong enough to pay the entropic cost of freezing of translational and rotational freedoms, and a guest with Lewis base is bound to the receptor with a fixed orientation. This could allow us to evaluate the hydrophobic interactions as an additional force, and this would eliminate interference from the compensation effects of translational and rotational entropy of guest. If we determine the thermodynamic parameters of binding of various Lewis bases with varying hydrophobicity, we can determine the contribution of hydrophobic interactions to the binding enthalpy and entropy.

The receptors have hydrophobic alkyl groups and the hydrophobic porphyrin core. These moieties are very non-polar so that the molecule is not soluble in water by itself. We attached poly(oxyethylene) groups at the terminals of the alkyl groups to solubilize the

receptors in water. Then the receptors have a unique structure where the non-polar environment is preserved in water. In such non-polar environment, Lewis acidic zinc can bind Lewis base and the Lewis acid-Lewis base interaction occurs without interference from water solvation. These receptors bind 4-alkylpyridines in the binding pocket as schematically shown in Figure 5. The binding constants, K/M^{-1} , as defined in equation (3) were determined by the electronic spectral changes in the Soret band upon addition of the guest in 0.1 M potassium phosphate buffer at pH 7 at 298 K.

$$K = \frac{[\text{receptor} \cdot \text{guest}]}{[\text{receptor}][\text{guest}]} \quad (3)$$

The free energy changes were calculated according to the equation:

$$\Delta G^\circ = -RT \ln K. \quad (4)$$

Fig. 4. Structures of receptors 1-3

The enthalpy changes and the entropy changes were determined by either isothermal calorimetric titrations or van't Hoff analysis of the binding free energy. For receptors **2** and **3**, both calorimetric experiments and the van't Hoff analysis based on the spectral changes were performed to give consistent results. Aggregation behavior of receptor **1**, however, prevented reliable measurements of isothermal calorimetry, so that only the thermodynamic parameters determined by the van't Hoff analysis were used. Receptor **1** and receptor **3** have poly(oxyethylene) groups with molecular weight of 750 at each terminal of the alkyl groups.

Fig. 5. Binding of alkylpyridines to receptors **1-3**

Fig. 6. Plot of the binding free energy of 4-alkylpyridines to **1**, **2**, and **3** against the number of CH₂ groups (n) in the guest. For **1**, $-\Delta G^\circ = (2.60 \pm 0.06)n + (21.2 \pm 0.2)$. For **2**, $-\Delta G^\circ = (2.75 \pm 0.06)n + (19.3 \pm 0.2)$. For **3**, $-\Delta G^\circ = (2.60 \pm 0.02)n + (21.78 \pm 0.6)$

Receptor **2** has poly(oxyethylene) groups with molecular weight of 350. Receptor **1** has only four alkyl chains to form the hydrophobic binding pocket while receptors **2** and **3** have eight

alkyl chains to form the hydrophobic binding pocket. These differences produced the different environment of the binding pocket and influences the mechanism of hydrophobic interactions significantly.

Fig. 7. Plot of $T\Delta S^\circ$ ($T = 298$ K) against ΔH° for binding of 4-alkylpyridines to **1**, **2**, and **3**. Arrows indicates the direction of changes as the length of the alkyl group, and thus the binding free energy, $-\Delta G^\circ$, increases. Bars indicate standard deviations

In Figure 6 are plotted the free energy changes of binding of 4-alkylpyridines to receptors **1**–**3** against the alkyl chain length of the guest. The binding free energy decreases *ca.* 2.6–2.8 kJ/mol per one CH_2 group, to confirm that hydrophobic interactions operate effectively. All these receptors bind alkylpyridines in a similar fashion in terms of free energy. In Figure 7 are plotted the enthalpy changes and the entropy changes for these host-guest pairs. The arrows indicate the direction of increasing alkyl chain length of the guest. The plot indicates that the binding mechanisms of alkyl groups recognition, i.e., hydrophobic interactions, are quite different among three receptors **1**–**3**. For receptor **1**, the alkyl group binding is driven by the enthalpic term: the longer alkyl groups are favored because of the exothermicity of the equilibrium. For receptor **3**, the alkyl group binding is driven by the entropic term: the longer alkyl group binding is favored because of the randomness of the system increases. For receptor **2**, both of the two terms, enthalpy and entropy, are favorable for longer alkyl groups.

The $T\Delta S^\circ - \Delta H^\circ$ plot in Figure 7 is discussed from the standpoint of enthalpy-entropy compensation. For receptors **1** and **3**, only weak enthalpy-entropy compensation was observed. The slopes are different from unity, indicating that the compensation temperatures are much different from room temperature. For receptor **2**, the negative slope revealed that both enthalpy and entropy drive the hydrophobic interactions. For receptor **1**

and **2**, the free energy changes were almost exclusively determined by the enthalpic term. For receptor **3**, the free energy changes were determined by the entropy term. The non-compensation was similar to the enthalpy-entropy changes in the transfer of alkane in water to liquid alkane, as discussed in Section 2.3.

In independent experiments, we evaluated the water accessibility to the binding pocket. The ratios of binding constants of pyridine to imidazole are listed in Table 1 for receptors **1-3**. Receptor **3** favors pyridine while receptor **1** does not disfavor imidazole as receptor **3**. Imidazole is hydrogen bonded to water while bound to the zinc since imidazole has two nitrogens. Therefore, a hydrophobic binding pocket where water cannot penetrate into the pocket should favor pyridine and disfavor imidazole. The water accessibility to the binding pocket is high for receptor **1**, medium for receptor **2**, and low for receptor **3**. The interpretation is also reasonable considering the structures of receptors: receptor **1** has only four alkyl chains so that water penetration into the binding cleft could be frequent, while receptor **3** has eight alkyl chains to protect the binding cleft from water penetration. For receptor **1**, the binding cleft could be too small to accomodate the long alkyl group of 4-alkylpyridines. Before the guest was bound, the alkyl groups of receptor **1** may be folded to avoid contact with water, but binding of 4-alkylpyridine caused conformational changes in the alkyl groups of receptor **1** to expose the non-polar surface of the alkyl groups to water to a larger extent than the uncomplexed receptor. This could induce the iceberg structure near the complex, by which enthalpy becomes negative.

	$K(4\text{-methylpyridine})/K(N\text{-methylimidazole})$
1	20
2	96
3	124

Table 1. Ratios of binding constants of pyridines to imidazoles as a probe for the polar/nonpolar environment of the binding pocket

The hydrophobic recognition of the guest alkyl group by receptors **1-3** seems similar in terms of free energy, but the enthalpic and entropic changes revealed that the binding mechanisms are quite different. For receptor **3** having well protected binding pocket, hydrophobic interactions are driven by an entropic force. This is classical hydrophobic interactions where water gains motional freedom upon released from the non-polar surface. In contrast, for receptor **1** having less protected binding pocket, hydrophobic interactions are driven by an enthalpic force. The origin of the enthalpic force can be either van der Waals interactions or the enhanced hydrogen bonding among water, i.e., iceberg formation, where more water can be exposed to non-polar surface upon binding of alkyl group. Receptor **2** with intermediate water accessibility binds the alkyl group by both enthalpy and entropy driving forces.

5. Perturbation approach to access hydrophobic interactions: Protein-ligand binding

5.1 Entropy-driven binding of an alkyl group to protein

We then discuss the binding of the alkyl group of *p*-alkylbenzamidine chloride to trypsin, (Talhout et al., 2003). Molecules with an amidinium group ($-C(NH_2)=NH_2^+$) are known to be an inhibitor of trypsin. The amidinium group of the ligand is bound to Asp189, Gly219, Ser190 and internal water through hydrogen bonding and the alkyl group is bound to a

hydrophobic pocket made by Trp215 and Leu199. The free energy changes, the enthalpy changes and the entropy changes are plotted against the carbon number in the alkyl group of the ligands in Figures 8-10, respectively. Figure 8 shows that the increase in the carbon number leads to the increased binding affinity, $-\Delta G^\circ$. As shown in Figures 9 and 10, enthalpy-entropy compensation was observed: the entropy term becomes favorable with increasing carbon number, while the enthalpy term becomes unfavorable. In this study, the binding of alkyl group to trypsin is driven by the entropic term.

Fig. 8. Plot of the free energy change in binding of alkylbenzamidine chlorides to trypsin at 25 °C at pH 8. Least squares line fit gave $-\Delta G^\circ = (-0.6 \pm 0.1)n - (24.1 \pm 0.4)$, where n is the carbon number of the alkyl group. Data were taken from Talhout et al. 2003

Fig. 9. Plot of the enthalpy change in binding of alkylbenzamidine chlorides to trypsin at 25 °C at pH 8. Least squares line fit gave $\Delta H^\circ = (1.5 \pm 0.4)n - (16.8 \pm 1.3)$, where n is the carbon number of the alkyl group. Data were taken from Talhout et al. 2003

Fig. 10. Plot of $T\Delta S^\circ$ in binding of alkylbenzamidine chlorides to trypsin at 25 °C at pH 8. Least squares line fit gave $T\Delta S^\circ = (2.1 \pm 0.3)n + (7.3 \pm 1.1)$, where n is the carbon number of the alkyl group. Data were taken from Talhout et al. 2003

Fig. 11. Plot of the free energy change in binding of alcohols to MUP-1 at 300 K at pH 7.4. Least squares line fit gave $\Delta G^\circ = (-3.87 \pm 0.26)n - (4.6 \pm 1.9)$, where n is the carbon number of the alkyl group. Data were taken from Malham et al. 2005

5.2 Enthalpy-driven binding of an alkyl group to protein

Binding of alcohols to the major urinary protein (MUP) was studied by Malham et al. (Malham et al., 2005). The primary hydroxy group of alcohol was hydrogen bonded to Tyr, and the alkyl group is bound to the hydrophobic pocket formed by Leu and Phe. The free energy changes, the enthalpy changes and the entropy changes are plotted against carbon number of alcohols in Figures 11-13, respectively. Figure 11 shows that the binding becomes

tighter as the alcohol is longer: the alkyl groups were bound to the protein through hydrophobic interactions. The driving force of binding was enthalpic, and the entropic term counteracted the enthalpy term, i.e., enthalpy-entropy compensation was again observed (see Figures 12 and 13). Malham et al. suggested that dispersive interactions between alcohol and the hydrophobic side chains of MUP are the origin of the negative enthalpy changes.

Fig. 12. Plot of the enthalpy change in binding of alcohols to MUP-1 at 300 K at pH 7.4. Least squares line fit gave $\Delta H^\circ = -(5.6 \pm 0.2)n - (13.8 \pm 1.4)$, where n is the carbon number of the alkyl group. Data were taken from Malham et al., 2005

Fig. 13. Plot of $T\Delta S^\circ$ in binding of alcohols to MUP-1 at 300 K at pH 7.4. Least squares line fit gave $T\Delta S^\circ = -(1.7 \pm 0.1)n - (9.2 \pm 0.8)$, where n is the carbon number of the alkyl group. Data were taken from Malham et al. 2005

Fig. 14. Plot of $T\Delta S^\circ$ ($T = 298 \text{ K}$) against ΔH° for binding of alkylbenzamidine chloride (filled triangle) or alcohols (filled circle) to proteins. Arrows indicates the direction of changes as the length of the alkyl group, and thus the binding free energy $-\Delta G^\circ$, increases.

6. Comparisons of thermodynamic parameters of binding of alkyl group to synthetic receptors and proteins

In Figure 14, values of $T\Delta S^\circ$ are plotted against ΔH° for both binding of alkylbenzamidine chloride and alcohol to proteins. The compensation temperature T_c was 224 K for the binding of alkylbenzamidine chlorides to trypsin and 986 K for the binding of alcohol to MUP. In the former case, the temperature at which the binding was studied is much higher than T_c so that the entropy term dominates the overall thermodynamics. In the latter case, the temperature at which the binding was studied is much lower than T_c , to result in the enthalpy driven binding. These two studies on the thermodynamics of binding of alkyl groups to protein demonstrate that the binding of the alkyl groups can be driven either by enthalpic or entropic driving forces, and T_c 's are much different from room temperature.

The compensation temperature of the binding of 4-alkylpyridines to receptor **1** was 800 K, and that of the binding to receptor **3** was 45 K based on the plot shown in Figure 7. The compensation temperature of the binding to receptor **2** was negative. These studies including both proteins and synthetic receptors demonstrated that T_c of binding of alkyl groups could be much different from room temperature, or even negative, and the origin of the diverse values of T_c should be the non-compensating properties of hydrophobic interactions.

7. Conclusion

The enthalpic and entropic contributions to the hydrophobic interactions were discussed. The transfer of alkane in the liquid state to water was driven by both enthalpic and entropic terms. The enthalpy-entropy compensation was not observed in the process. Binding of an

alkyl group to synthetic receptors with varying degrees of water accessibility to the binding pocket showed that the hydrophobic interactions between the alkyl group of the guest and the alkyl chains of the receptor were driven by the entropic term if the binding pocket was sequestered from water and by the enthalpic term if the binding pocket was exposed to water. For the binding of the alkyl group to the synthetic receptors, strong enthalpy-entropy compensation was not observed. We suggest that hydrophobic interactions are driven by both enthalpic and entropic driving forces, and the relative contribution to the free energy was determined by the water accessibility to the binding cleft. Thermodynamic data for the binding of alkyl groups to proteins also showed much diverse compensation temperatures, 224 K for one case and 986 K for the other case. Non-compensating enthalpy and entropy may be a general property of hydrophobic interactions.

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Application of Thermodynamics to Biological and Materials Science

Edited by Prof. Mizutani Tadashi

ISBN 978-953-307-980-6

Hard cover, 628 pages

Publisher InTech

Published online 14, January, 2011

Published in print edition January, 2011

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Mizutani Tadashi (2011). Thermodynamics of Supramolecular Structure Formation in Water, Application of Thermodynamics to Biological and Materials Science, Prof. Mizutani Tadashi (Ed.), ISBN: 978-953-307-980-6, InTech, Available from: <http://www.intechopen.com/books/application-of-thermodynamics-to-biological-and-materials-science/thermodynamics-of-supramolecular-structure-formation-in-water>

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