Entropic Trapping Binding Mechanism: Its Likely Role in Receptor−Ligand and Other Biochemical Systems†

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Entropy-driven binding continues to be discovered in receptor−ligand systems as well as in other important biochemical systems. In receptor−ligand systems the "thermodynamic agonist−antagonist discrimination" is often found: in some such receptor types the binding of antagonists is entropy-driven, in others this is a characteristic of the agonist binding. The interpretation of the entropy-driven binding mechanism in the systems in question is still rather elusive. Experimental findings clearly indicate that the entropic binding mechanisms which are usually considered cannot provide a consistent interpretation in these cases. Entropic trapping was therefore proposed in our earlier papers as a possible binding model which does not contradict the experimental facts. It is pointed out here that through the work which has appeared subsequently the existence of such a mechanism has been firmly established and that its role is strongly corroborated by the more recent data from several biochemical systems.

INTRODUCTION

The binding in a number of important biochemical systems has been found to be entropy-driven (i.e., the entropy term overcompensates the enthalpy term) and moreover is often characterized by the enthalpy and entropy changes $\Delta H°$ and $\Delta S°$ with the properties

$$|\Delta H°| \sim 0 \text{ and } \Delta S° > 0$$

It is notable that such a binding typically takes place in a hydrophobic, sterically constrained environment. Among the important examples are as follows:

(1) The binding of ligands to membrane receptors. In recent papers by Borea et al.[1] and by Maksay and McKernan,[2] available thermodynamic data are examined for the binding of agonists and antagonists to five G-protein coupled receptors (GPCR): β-adrenergic (β-AR), adenosine A_1, adenosine A_{2A}, dopamine D_2, and serotonin 5-HT_{1A}, and for the binding to four ligand-gated ion channel receptors (LGICR): glycine, γ-aminobutric acid ($GABA_A$), serotonin 5-HT_3, and nicotinic acetylcholine receptor (nAChR). Among these receptor systems, all LGICR and three GPCR (β-AR and adenosine A_1 and A_{2A}) show "thermodynamic agonist−antagonist discrimination", that is, in some of these receptor types the agonist binding is entropy-driven, in others it is the binding of antagonists that is entropy-driven.

(2) The binding of reduced nicotinamide adenine dinucleotide (NADH) to horse liver alcohol dehydrogenase (LADH).[3]

(3) The binding of coenzyme NADH to octopine dehydrogenase (ODH).[4]

(4) The binding of anesthetics to two Ca^{2+}-ATPases.[5]

The above list of systems where the entropy-driven binding was found, with the characteristic properties as referred to below, is by no means complete. We limited ourselves here only to several specific examples, to keep the focus.

Explaining the mechanism of the entropy-driven binding in the systems in question has remained a challenge. The more recent experimental work, however, has made it possible to evaluate various hypotheses which led to the proposing of a novel binding mechanism, entropic trapping by Miklavc et al.,[6,7] consistent with the experimental findings. It is shown here that, through the work which appeared subsequently, the existence of such a mechanism has been firmly established and that its role is strongly corroborated by the more recent data from several biochemical systems.

THE $\Delta S° > 0$ BINDING MECHANISM

The Role of Hydrophobic Interactions, Large Scale Conformational Changes, and Displacement of Water Molecules. Hydrophobic effects, large scale conformational changes, or displacement of water molecules are often suggested as possibly leading to the entropic binding in the above systems. These assumptions cannot be sufficient to explain the corresponding experimental findings, for the following reasons:

(1) The dissociation constant k_D of the NADH−ODH complexes changes by about 10%[4] and that of several β-AR antagonists by less than 50% over the temperature change from 2 to 35 °C.[8,9] Moreover, the corresponding van't Hoff plots are essentially linear.

$\Delta H°$ is thus temperature-nearly-independent which cannot be reconciled with a hydrophobic origin of the binding mechanism.[10]

(2) It is found that the binding of most ligands to membrane receptors seems to occur with null or minimal

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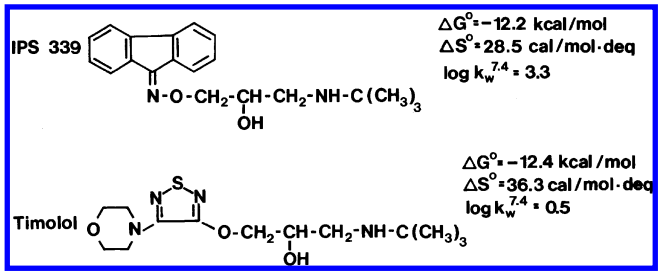

Figure 1. $\Delta G°$ and $\Delta S°$ in the entropy-driven binding of potent antagonists IPS 339 and timolol to the β-adrenergic receptor at 37 °C.[9] $k_W^{7.4}$ is an experimentally determined lipophilicity index. The value of this index is notably different for the two drugs which otherwise bind with a similar $\Delta S°$.

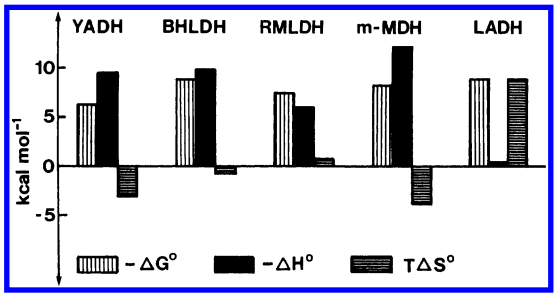

Figure 2. Diagrammatic representation of $\Delta G°$, $\Delta H°$, and $\Delta S°$ of the NADH binding to dehydrogenases at 25 °C and pH 7.6[3]. YADH: yeast alcohol dehydrogenase; BHLDH: beef heart lactic dehydrogenase; RMLDH: rabbit muscle lactic dehydrogenase; m-MDH: pig heart mitochondrial malate dehydrogenase; LADH: horse liver alcohol dehydrogenase.

specific heat $C_p°$ changes,[11] at variance with other processes involving biological macromolecules. Such low $\Delta C_p°$ values could not be reconciled with a dominant role of hydrophobic interactions in the binding mechanism.

(3) It was found[9] that $\Delta H°$ and $\Delta S°$ in the entropic binding of antagonists to β-AR do not correlate ($r = 0.6$) with the experimentally determined lipophilicity index log k_W.[7.4] Two of the most potent antagonists, IPS 339 and timolol, e.g., bind with similar entropy increases but differ very much in the lipophilicity index (Figure 1).

(4) NADH binds with $\Delta S° > 0$ to LADH; its binding to the other dehydrogenases, however, is enthalpy-driven[3] (Figure 2). It is difficult to assume that hydrophobic effects in the binding of the same ligand to structurally and biologically very similar systems would be so different.

(5) The entropy-driven binding was found also in systems where ligands were dissolved in organic solvents where hydrophobic interactions are absent.[5]

(6) X-ray studies revealed[12] that no large scale structural changes occur upon the binding of H_2NADH to LADH which is purely entropy-driven. The net of water molecules in the binding cleft remained intact on the binding. No water molecule was displaced.

From the above observations we may conclude that a mechanism of the binding driven by $\Delta S° > 0$ should exist which is not based on hydrophobic effects, or large scale conformational changes, or displacement of water molecules.

Binding by Entropic Trapping. As noted already, van't Hoff plots which have been reported for the systems in question are linear and essentially no changes in the specific heat $C_p°$ upon binding were found in most cases of the receptor−ligand systems where $\Delta C_p°$ was measured. Since the solvation/desolvation processes are generally associated with the curved van't Hoff plots and a nonzero $\Delta C_p°$ we have to conclude that, for some reason, the free energy contributions due to the changes in the solvation on binding apparently are absent in the binding constants observed in these systems. It is thus a burning question as to why effects of the solvation changes are not observed as expected.

It is not generally recognized that one may not know a priori equilibrium of which process in the system is reflected in a measured binding constant when a particular method is used. Generally, the reason for this uncertainty is in the, possibly large, differences in the characteristic time scales of the processes involved.[13] The striking result that thermodynamic quantities determined for the same system by different approaches were found to be different[14] may well have the same roots, as will be seen more clearly later.

As pointed out some time ago by Leysen and Gommeren,[15,16] the binding data have been usually interpreted on the basis of the law of mass action which, strictly speaking, is only applicable to interactions between the molecules in solution and takes into account the molecular concentrations of reagents and reaction products. The conditions for its applicability are not likely to be fulfilled in the usual complex physicochemical receptor−ligand interaction, say in a membrane or protein. In such systems "surface phenomena" pointed out by Blank[17] are likely to occur. The term surface phenomena should here be regarded as a vague description of the complex interaction of ligands with the binding sites on the surface of a membrane, or within the membrane interior, or on a macromolecule. The states of a ligand in the "surface region" should be adequately and conveniently discussed on the basis of the free energy landscape model.[18] Elementary statistical thermodynamics underlying this model will also be adequate for our present analysis.

Entropy-driven binding of interest here characteristically takes place in hydrophobic, sterically constrained environments, e.g., in hydrophobic transmembrane channels or protein clefts. It was found recently by Chiara et al.[19] that 3-(trifluoromethyl)-3-(*m*-iodophenyl)diazirine (TID), a hydrophobic resting-state inhibitor of nAChR, equilibrates with the nAChR membrane's lipid in less than a millisecond. In contrast, equilibration with the channel region of the receptor where the binding site is located was more than 20 times slower. Furthermore, whatever the restricted pathway along which TID gains access to its binding pocket there is no significant potential energy minimum for TID-protein interaction in this region other than the pocket bound by the three residues δLeu-265, δVal-269, and δLeu-272. It was also found that the majority of the TID (>99%) ends up in the membranes. In a study of volatile anesthetic halothane binding domains on nAChR[20] saturable binding to both the native *Torpedo* membranes and the isolated nAChR was observed. The data suggest several binding domains for halothane in the transmembrane region of the nAChR. Similarly it was found[21] that halothane binds saturably to skeletal muscle sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA1) membranes and that 38−56% of halothane incorporates into SERCA1 and 38−53% into membrane lipids. The data indicate heterogeneous halothane binding, presumably in discrete sites in the enzyme.

As far as we know, detailed binding studies of the kind quoted above are still rather scarce and are hardly present in earlier experimental work on the systems in question. We

may assume, on the basis of admittedly scarce experimental evidence, that in these systems equilibration of ligands with a lipid is generally much faster than equilibration of ligands inside the membrane with their binding site in the transmembrane channel and, moreover, that the binding site is not in direct contact with bulk water. It follows then that a binding constant measurement may in fact reflect a "surface process", i.e., it may reflect the equilibration of the ligands between the binding sites and their neighborhoods inside the membrane. In the corresponding free energy change $\Delta G°$ the contribution due to the desolvation of ligands would clearly cancel: if $\Delta G°(s \rightarrow m)$ is the free energy change corresponding to the transfer of the ligand from the solvent to some "average standard state" in the membrane interior and $\Delta G°(s \rightarrow b)$ is the free energy change corresponding to the transfer of the ligand from the solvent to the binding site inside the membrane, we have

$$\Delta G°(s \rightarrow b) = \Delta G°(s \rightarrow m) + \Delta G°(m \rightarrow b)$$

where $\Delta G°(m \rightarrow b)$ corresponds to the transfer from the average standard state to the binding site in the membrane interior. According to the characteristic time scales and ligand distributions assumed on the basis of the discussion above the observed free energy of binding $\Delta G°_{obs}$ equals to

$$\Delta G°_{obs} = \Delta G°(s \rightarrow b) - \Delta G°(s \rightarrow m) = \Delta G°(m \rightarrow b)$$

This could thus be a plausible reason as to why the observed van't Hoff plots are linear and why hardly any change $\Delta C_p°$ in specific heat upon binding was found, in other words, why the desolvation is not reflected in the temperature dependence of the binding constant. It should be noted that the changes of the thermodynamic quantities that are observed on binding may depend on the experimental procedure. If, e.g., enthalpy change $\Delta H°$ is determined calorimetrically the results could reflect also effects due to changes in the solvation. On the other hand, if a spectroscopic method is used the result may reflect equilibration within a "surface", with the effects of the desolvation canceled out, as pointed out above. It may perhaps be possible to explain the discrepancies in the experimentally determined enthalpy changes found by Naghibi et al.[14] on this basis, after a more detailed investigation of the processes involved.

With the above observations in mind, the binding data of, say, β-AR ligands may be rationalized by assuming that upon reaching the binding pocket deep in the transmembrane channel by a diffusion process, a β-agonist forms a tight "normal" bond ($\Delta H° < 0$, $\Delta S° < 0$), but a β-antagonist cannot form a tight bond ($|\Delta H°| \sim 0$) because of the structural properties. By $\Delta H°$ and $\Delta S°$ the enthalpy and the entropy change per molecule are denoted here, measured from an "average constrained state" in the membrane interior which can be taken as a standard state. Due to the looseness of the bond of the ligand in the binding pocket the phase space of rotations/internal rotations "opens up", leading to

$$\Delta S_r° > 0$$

This entropy increase drives the binding in the systems in question. The changing of the $\Delta H°$ and $\Delta S°$ along the diffusion path reaching the binding pocket may be qualitatively presented as in Figure 3. There is some experimental

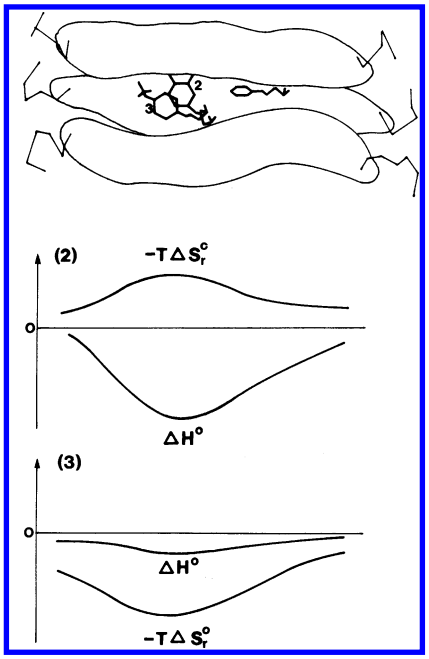

Figure 3. Schematic presentation of $\Delta H°$ and $\Delta S°$ along a transmembrane channel in the enthalpy-driven (diagram 2) and the entropy-driven (diagram 3) binding of ligands in the channel (entropic trapping). Note that $\Delta H°$ and $\Delta S°$ are defined here as the enthalpy and the entropy changes of a molecule with respect to an average constrained state in the membrane interior.

evidence corroborating this picture reported by Tota and Strader.[22] The site of the binding of the fluorescent antagonist carazolol to β-AR was found to be more than 10 Å deep below the membrane surface. Moreover, the fluorescence lifetime of carazolol bound to β-AR (8.4 ns) was found to be similar to the lifetime in free carazolol (8.0 ns) in organic solvents which is in agreement with the assumption of a rather loose bond at the β-AR binding site. It should be mentioned that, in the case of a small difference in free energy of the two binding modes described above, it can happen that a particular ligand would bind partly in the enthalpy-driven mode (2) and partly in the entropy-driven mode (3) depicted in Figure 3. In the β-AR case, e.g., this situation may arise in the binding of the partial agonists. The values of $\Delta H°$ and $\Delta S°$ corresponding to the binding of the partial agonists in these systems were found to be between those of the agonists and the antagonists.[8]

The existence of the entropic trapping binding mechanism proposed originally on hypothetical grounds[6,7] and the behavior of the thermodynamic quantities as depicted schematically in diagram (3) of Figure 3 have been confirmed already by an analysis of a very simple system: the diffusion of a mass point particle along a rigid channel of a circular cross section, with a radius that varies along the channel axis.[23] $\Delta H° = 0$, because of the rigid wall, but entropic trapping could be observed in the region of increased cross section, in this case due to an increase of the translational entropy. Moreover, the existence of the entropic trapping mechanism has been established in computer experiments on the diffusion of polymers in random environments.[24] The surface in this case was a two-dimensional matrix of rigid obstacles, and polymers were assumed to consist of flexibly bound rigid subunits of equal length which was comparable to the distance between adjacent obstacles. Simulations were performed with polymers of equal length, in most cases

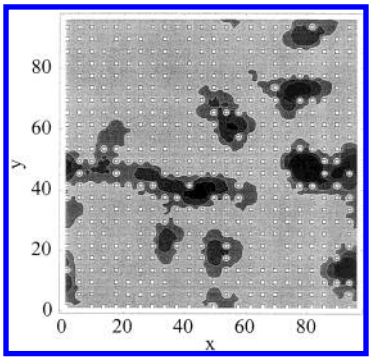

Figure 4. Plot showing the probability density for finding a polymer at a particular site within a lattice of obstacles (single white squares). At the darker sites the probability density is increased because of the cavities formed by the removal of the obstacles. *The density at these sites is increased because of the entropic trapping. There are no attractive potentials in the systems.*[24] Reprinted with permission from Slater, G.; Wu, S. Y. *Phys. Rev. Lett.* **1995**, *75*, 164−167. Copyright 1995 by the American Physical Society.

chosen to be near 10 subunits. When in a uniform matrix, consisting of obstacles cavities were created by a removal of obstacles, the probabilities of finding a polymer at the sites of such cavities increased, due to an increase of the conformational entropy $S_c°$ with respect to the regular parts of the matrix (Figure 4). Since no attractive forces are present in the systems, the "binding" is purely entropy driven, i.e., due to $\Delta S_c° > 0$ at the site of the cavity. It should be stressed that the physical origin of the binding mechanism is the same in the two examples discussed above—it is the entropy increase at the binding site. Clearly seen are advantages of the free energy landscape approach for discussing the binding in such complex systems.

ILLUSTRATIVE EXAMPLES

The two examples presented above show that the entropic trapping binding mechanism does in fact exist. It, however, still remains to be shown whether this is the mechanism of entropy-driven binding in the biological systems in question. The experimental findings pointed out earlier in this paper led to the conclusion that there should be a mechanism of the entropic binding basically different from the mechanisms that are usually considered. The entropic trapping so far appears to be consistent with the available experimental information. A direct confirmation of the mechanism in a particular biological system, however, would still be very difficult at present. Computational simulations of such large biological systems could not be carried out yet for a sufficiently long time interval.

Studies of mobility at the binding site and in its neighborhood should provide crucial information but are likely to be very demanding. One may thus attempt to draw conclusions by comparing the binding data of structurally similar molecules, as it is often done in structure−activity relationship studies. The difficulty in this particular case is that ligands that bind entropically often exhibit considerable structural diversity, as evidenced, e.g., by β-AR antagonists.[9] The most complete experimental studies appear to have been reported on the entropy-driven binding of anesthetics halothane and propofol (Figure 5) to two Ca^{2+}-ATPases, plasma membrane Ca^{2+}-ATPase (PMCA), and SERCA1, vital in

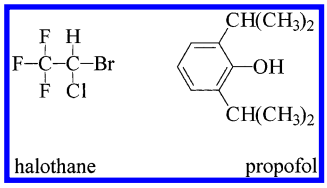

Figure 5. Anesthetics used in the studies of the binding to Ca^{2+}-ATPases PMCA and SERCA1.[5]

cellular Ca^{2+} regulation. We again stress that, in addition to the binding constant studies in the two biological systems, the distribution of halothane was investigated in the case of SERCA1: 38−56% of halothane was found to incorporate into SERCA1 and 38−53% into the lipids. The binding constant measurements could thus be assumed to reflect equilibration at the sites inside the membrane including the enzyme. This should thus be a clear case of binding within a "surface", in the generalized sense discussed above. It should be stressed that the entropy-driven binding of halothane was qualitatively similar when the ligand was dissolved in water, dimethyl sulfoxide (Me_2SO), *N*-methylformamide (NMF), and *N*,*N*-dimethylformamide (DMF). Propofol was dissolved only Me_2SO. The analysis of the binding data was performed by two theoretical approaches which indicated consistently that the binding to the enzyme was entropy-driven. According to the experimental results[5] at 25 °C in PMCA $\Delta\Delta H° \equiv \Delta H°$(propofol) − $\Delta H°$(halothane) = −0.1 ± 1. kcal mol^{-1} K^{-1} and $\Delta\Delta S° \equiv \Delta S°$(propofol) − $\Delta S°$(halothane) = 5. ± 2.5 cal mol^{-1} K^{-1}. In SERCA1 the corresponding results are $\Delta\Delta H° = 0.6 \pm 0.8$ kcal^{-1} K^{-1} and $\Delta\Delta S° = 9. \pm 2.$ cal mol^{-1} L K^{-1}. In each case the binding was characterized by $\Delta S° > 0$. According to the entropic trapping model the entropy increase should arise due to the increase of the ligand phase space at the binding site. The larger $\Delta S°$ in the case of propofol should thus be due primarily to the contribution of the internal rotations of the two $-CH(CH_3)_2$ groups. Experimental values of entropy increase due to "unblocking" of an intenal rotation are in the range[25] of 2.7−4.3 cal mol^{-1} K^{-1} which agrees well with the above quoted experimentally established values of entropy increase. It was conjectured[5] that the binding cavity could possibly be hydrated and the entropy increase might reflect the desolvation of the cavity on binding. The bigger entropy increase observed for propofol should mean displacement of more water molecules. The difficulty with this view is that the entropy increase due to the release of a single water molecule should have values[26] between 10 and 25 cal mol^{-1} K^{-1} which are considerably larger than the difference in entropy increase between propofol and halothane. Moreover, no evidence is available supporting the assumption of a hydrated binding cavity in these particular systems.

If ligands are larger polyatomic molecules of considerable structural diversity, like, e.g., β-AR antagonists, the data on the entropy-driven binding generally become more difficult to rationalize. One would actually have to model receptor−ligand states along the transmembrane channel which could easily lead to problems that we cannot handle adequately yet. Otherwise plausible explanations of the binding data are possible rather exceptionally. In the case of β-AR antagonists IPS 339 and timolol (Figure 1), e.g., the similarity of the structures appears to be sufficient to allow a quantitative explanation of the difference in the entropy increase on binding, using essentially the same arguments as in the case

of anesthetics. Since internal rotations of the 3-cyclic moiety in IPS 339 can be assumed to be hindered, because of the large size of this group, the number of "active" internal rotations in timolol is by two greater than in IPS 339. With the experimental values of the entropy increase due to "unblocking" of an internal rotation, which, as quoted above, are in the range of 2.7 − 4.3 cal mol^{-1} K^{-1}, the experimental difference $\Delta S°$(timolol) − $\Delta S°$(IPS 339) = 7.8 cal mol^{-1} K^{-1} can be easily accounted for.

CONCLUSIONS

In conclusion we may say that in the systems considered the entropic trapping mechanism provides an interpretation of the entropy-driven binding which is consistent with the available experimental information. The existence of this binding mechanism has been firmly established by now, but its role seems not to have been duly recognized, particularly in pharmacological and biochemical studies. It should generally be present, although not necessarily dominating, in the binding processes inside sterically constrained "random" environments, and it appears to allow considerable structural diversities of the ligands. It could also be expected that the binding cavity may be changed in certain ways while retaining the binding capacity for the same ligands. The mechanism in question should thus deserve particular attention also when dealing with systems where the binding cavities change, e.g., due to mutations.

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