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Conformational Flexibility and Receptor Interaction

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Abstract—This theoretical analysis shows that the experimentally observed standard Gibbs free energy of binding of a ligand by a receptor can be described by two terms. One term describes the free energy of binding of the drug to the receptor when both are in their lowest energy conformation. The second term gives the difference between the average and the lowest conformational energy of the two species involved. It also follows that all drug molecules having an energy higher than the minimum energy, must have a higher affinity than molecules occurring in the minimum energy conformation, independent of the energy level of the receptor bound conformation.

Introduction

Protein–ligand interactions are essential for many biological processes. This molecular recognition step is characterized by the formation of mostly non-covalent bonds, which involve physical contacts between the ligand and its receptor. Insight into the structural factors of the small ligand and the protein, in the energetics (thermodynamics), in the dynamics (kinetics) are necessary for a full understanding of this process. Reviews on these aspects have been published recently.[1–3]

Of particular interest is the conformation of the ligand bound to the receptor (often named the bioactive conformation) with respect to the conformations the ligand may assume when free in solution. It has been shown[4] that protein-bound ligands may occur in conformations far above the global minimum and, in many cases, not even in any local minimum. How can we describe the relationship between an experimentally observed ligand–protein binding constant, conformational flexibility of the ligand in solution, and the receptor-bound conformation in thermodynamic terms?

Key words: Conformational flexibility; receptor interaction; receptor bound conformation; free energy of binding; conformational energy.

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Conformation and Receptor Binding

The reaction to be discussed is that between a ligand L and a receptor (protein) R, which can be described as

$$L + R \rightleftharpoons LR \quad (1)$$

If the measured affinity (association) constant of this reaction is given by K, then the corresponding standard Gibbs free energy change follows from $\Delta G^0 = -\mathrm{RT}\ \ln K$. Note that a higher affinity is associated with a higher value of K, and with a correspondingly more negative value of ΔG^0. Instead of the association constant, a dissociation constant may be used to describe eq (1). Then evidently a stronger binding is related with a more positive value for ΔG^0. The difference is not essential, but should be noted.

It is known that the ligand may assume several conformations in solution. Each conformation has an energy, so this results in a continuous Boltzmann distribution over a large range of energy levels. The result of many calculations are frequently restricted to a list of discrete energy levels such that every conformation is associated with an energy level. These levels will be indicated by i = 0, 1,. . .n. Different conformations can have the same energy but we will not concern ourselves with these at this stage. All ligand molecules having the same energy will be given the same number, thus L_i ($i = 0$, 1,. . .n) represents one of the possible conformations of a

ligand having an energy $E(L_i)$ (kcal mol^{-1}). These energies have increasing values indicated by $E(L_0)$, $E(L_1)$,..., $E(L_n)$. L_0 is named the minimum energy or lowest energy conformation. Note that energy is required to take molecules from a low level to a higher energy level, and consequently that energy is released when molecules go from a high energy level to a low energy level. When we talk about energy and energy levels of the isolated ligand, what kind of energy do we mean? Ajay and Murcko[1] pointed out that the term 'energy' is used loosely by different authors. They argued that in solution conformational energy may be equated to conformational enthalpy. Also Williams et al.[5] assumed that an enthalpy term may be used to describe energy differences between conformational states.

Suppose that it we could measure the reaction between 1 mol of L occurring in the lowest energy conformation and 1 mol of the receptor R

$$L_0 + R \rightleftharpoons LR \quad (2)$$

The standard Gibbs free energy ΔG_0^0 of this association reaction should follow from the measured association constant: $\Delta G_0^0 = -RT \ln K_0$. Note that the subscript zero indicates that we are discussing the lowest energy conformation, whereas the superscript zero refers to the standard state. Energetically, this reaction includes enthalpy and entropy terms. This implies that from a structural point of view, hydrogen bonds, other non-covalent interactions, solvent influences and other structural changes of both ligand and receptor have had their effect. These aspects are discussed elsewhere.[1–3,5] Let us next consider the binding of a higher energy conformation L_i. The energy involved in this binding process can be calculated by first converting 1 mol of L_i from its energy level i to the lowest energy level. During this step an amount of energy equal to $E(L_0)$–$E(L_i)$ is released. In the next step the binding of 1 mol of L_0 to R takes place which proceeds as discussed above. In other words for the reaction

$$L_i + R \rightleftharpoons L_0 + R \rightleftharpoons LR \quad (3)$$

the standard free energy change is given by

$$\Delta G_i^0 = \Delta G_0^0 + E(L_0) - E(L_i) \quad (4)$$

Because ΔG_i^0 is more negative than ΔG_0^0, high energy conformations bind more strongly than low energy conformations. However the binding of L_i can not be studied experimentally because molecules with different conformational energies are distributed over the several energy levels according to statistical mechanics. This limits the probability of having a large fraction of molecules in high energy conformations. We will illustrate this using an example from the literature.[6]

Ketanserin is a conformationally flexible 5-HT antagonist. Results of a semi-empirical quantum-chemical calculation, considering rotation around 3 torsion angles, are given in Table 1. Conformations were obtained by considering torsional angle steps of 10 degrees, resulting in $36^3 = 46656$ conformation calculations. It can be seen that 116 conformations have a (relative) energy in the range 0–1 kcal mol^{-1}, 695 different conformations are found in the range 1–2 kcal mol^{-1} etc. The question now arises, how are the molecules distributed over these conformational energy levels? From statistical mechanics, the fraction f_i of molecules found in a conformation energy level i is given by the Boltzmann distribution law (eq (5)).[7]

$$f_i = g_i \exp(-E(L_i)/RT)/\Sigma_i g_i \exp(-E(L_i)/RT) \quad (5)$$

in which $E(L_i)$ has the meaning given above, R is the gas constant and T the absolute temperature; g_i indicates the degeneracy of an energy level (i.e. $g_0 = 116$ in Table 1). Equation (5) may be used to calculate the distribution of ketanserin molecules over the energy levels. The results expressed as f_i values are also given in Table 1. Note that, according to this calculation, the major fraction is not found in the lowest energy level. On the other hand 94% of the molecules are found in the energy range 0–3 kcal mol^{-1}. It is also seen that the high energy conformations have a low probability.

Table 1. Some conformational energy distribution properties of ketanserin

Energy level	Range (kcal mol^{-1})	No. of conformations	$g_i \exp(-E(L_i/RT)$	f_i	$f_i E(L_i)$ (kcal mol^{-1})
0	0-1	116	49.8	0.365	0.183
1	1-2	695	55.2	0.404	0.609
2	2-3	1602	23.5	0.172	0.430
3	3-4	2448	6.6	0.049	0.168
4	4-5	2493	1.2	0.009	0.041
5	5-6	2132	0.2	0.001	0.006
6	6-7	1439			
7	7-8	1397			
8	8-9	1361			
9	9-10	1220			
10	10-20	8305			
11	> 20	23448			
Summation		46656	136.5	1.00	1.436

The lowest range 0–1 has been given the number 0 etc. The number of conformations found in the several ranges have been taken from Tollenaere.[6] RT has been given the value 0.592 kcal mol^{-1}. For $E(L_0)$, $E(L_1)$ the values 0.5, 1.5 etc have been taken.

Although the calculations relate to an in vacuo situation, the same principles hold in solution. It is also important to note that (according to 5) the distribution of molecules over the several energy levels (that is, the value of f_i) is independent of the concentration. In other words, this distribution is not influenced by the presence of receptors. This means that in the experimental process of binding of L to R, the fractional distribution over the several energy levels remains constant. Therefore the binding of 1 mol of L as described by the overall eq (1) means that f_0 mol of L_0, f_1 mol of L_1, etc are bound. The amount of energy involved in the binding of f_i mol of L_i to R is given by $f_i\ \Delta G_i^0$ (with ΔG_i^0 given by 4). The binding of one mol of L is therefore given by $\Sigma_i f_i \Delta G_i^0$, or

$$\Delta G^0 = \Sigma_i f_i \Delta G_i^0 = \Sigma_i f_i \Delta G_0^0 + \Sigma_i f_i E(L_0) - \Sigma f_i E_i(L_i) \quad (6)$$

By definition $\Sigma_i f_i = 1$ and $\Sigma_i f_i E(L_i)$ is equal to the average energy of the free ligand molecules, which can be indicated by $E(L)_{av}$. Then 6 can be written as

$$\Delta G^0 = \Delta G_0^0 + E(L_0) - E(L)_{av} \quad (7)$$

We note that the observed free energy of binding is more negative (the binding is stronger) than the free energy of binding of the lowest energy conformation. In the example of ketanserin $E(L_0)-E(L)_{av}$ amounts to $-1.4\,kcal\,mol^{-1}$.

Discussion

We have shown that higher energy conformations have a higher affinity for a receptor than lower energy conformations, independent of the conformation in which the ligand is bound to the receptor. This conclusion has no further practical application because this binding can not be realized experimentally. On the other hand, it is be necessary to state this with some emphasis because frequent remarks can be found in literature which suggest the opposite. We agree with the statement of Pettitt and Karplus[8] that: 'the mean interaction energy is not that associated with the minimum energy structure, nor even the average structure as obtained from an X-ray analysis'. This statement has also been reinforced by Ajay and Murcko[1] when they remark that ensemble averages should be used. They also state that the most common assumption is that these averages are replaced by values corresponding to a single stable structure.

In this paper we have tried to correlate microscopic interaction energies with macroscopic, measurable thermodynamic quantities. We have focused on the conformational energy distribution of the (small) drug molecule, but of course, the receptor may also occur in more than one conformational state. When this is taken into account, 2 should be modified to

$$L_0 + R_0 \rightleftharpoons LR \quad (8)$$

This will result in an expression equivalent to 7 but now including a term for R:

$$\Delta G^0 = \Delta G_0^0 + E(L_0) - E(L)_{av} + E(R)_0 - E(R)_{av} \quad (9)$$

For the ketanserin example described above, $E(L_0)-E(L)_{av}$ was found to be $-1.4\,kcal\,mol^{-1}$. The question remains to be answered how this number is influenced by the solvent. At this moment we don't have an indication about the order of magnitude of the factor $E(R_0)-E(R)_{av}$.

In our analysis we did not need to assume anything about the energy or conformation of the receptor bound drug. The term $E(L_0)$–$E(L)_{av}$ is independent of the difference in energy between the lowest conformation and the receptor bound conformation. This latter kind of energy difference is included in the term ΔG_0^0, and the intriguing question remains to be solved how this ΔG_0^0 is related to properties of the drug and the receptor. This question is also relevant in considering the activity of a series of (related) compounds, where it may be assumed that differences in activity are more likely to be caused by differences in ΔG_0^0 than by differences in $E(L_0)-E(L)_{av}$ because it may be expected that (related) ligand molecules in solution have a similar average energy.

The fact that ligand molecules in solution have different energies does raise the question how the kinetic behaviour of these conformers is related. The simplest view is that the activation energy is lower for the high energy conformers, resulting in faster kinetics for these species. When the interconversion rate between conformations is fast relative to the kinetics of ligand interactions, it can be shown that this results in an observed activation energy equal to the activation energy of the lowest energy conformation and that the observed pre-exponential factor has increased.

Molecular recognition and interaction is a fascinating process, and we are still far from understanding it. The hope is that this paper will stimulate discussion especially on the contribution of individual conformers to experimentally observable quantities.

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