THE ROLE OF HYDROGEN BONDING IN DRUG-RECEPTOR INTERACTIONS*

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ABSTRACT

Amongst the usual components of free energy of interaction between molecules, particular hydrogen bonds may be crucial for the specificity of interaction. Without information on the receptor structure, it is, however, difficult to single out the role of hydrogen bonding from that of other components. This problem will be illustrated by examples of various drugs and corroborated by energy analysis of a simple computational model of receptor-drug interaction. The model permits conjecture about a possible receptor triggering mechanism involving proton transfer.

INTRODUCTION

Specific interactions between messenger molecules, such as hormones and neurotransmitters (drugs or ligands in general terms), and their receptor dominate the chemical regulation of biological processes. The regulation mechanism may be resolved into consecutive stages the first of which is termed recognition of the ligand by the receptor, or binding, which is a reversible reaction. This stage is followed by a conformational rearrangement of the initial complex and later by certain biochemical reactions which are characteristic of the receptor type. The separation of the mechanism into distinct stages is somewhat artificial but is convenient in introducing two general types of drug activity, agonists and antagonists. The binding of the former elicits the complete receptor mechanism whereas binding of the latter is limited to this stage and thus blocks eventual agonist activity. Both types of drug are important for medicinal applications.

Binding of ligands, expressed as the change of free energy, is determined by intermolecular forces which include hydrogen bonding as a special case. Entropy effects are often neglected in considering the specificity of interactions although they may be determining differences in drug affinity [1].

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Although thermodynamic parameters of drug–receptor interactions can readily be determined, the molecular determinants of the binding mechanism can be constructed for most of the pharmacological receptors only from considerations of structure–activity relations established on a series of ligands binding to the same receptor type because the receptor structure is not known at the atomic level. Singling out the role of particular hydrogen bonds from that of other types of energy-yielding, intermolecular interactions is not straightforward and the mere presence of H-bonding groups in active molecules often needs additional evidence to show that these groups are indeed utilized in H-bonding. The situation is much more favourable for drug receptors of known structure, e.g. enzymes and DNA. A good example of well-defined interactions is dihydrofolate reductase and its inhibitors where X-ray diffraction structures clearly demonstrate concrete H-bonds [2,3]. The introduction of additional H-bonding capacities in the inhibitor trimethoprim design has led to drugs with superior affinity [4]. The recent free-energy perturbation study [5] of the binding of another inhibitor, methotrexate, to dihydrofolate reductase mutants is very instructive in demonstrating the contributions to the free energy of several amino acid residues interacting with the inhibitor. Although most of the energy is due to hydrophobic interactions, it is a salt bridge between the inhibitor and an aspartate of the enzyme that determines the specificity of binding although it contributes only \( \sim 1.8 \text{kcal mol}^{-1} \) to binding. H-bonding patterns defining the specificity of interaction have been proposed for antiviral antitumor antibiotics of the netropsin type for binding to DNA [6] as well as for ristocetin antibiotics topically complexing with bacterial cell-wall fragments [7].

Participation in receptor–drug complexation is but one of the roles that H-bonding assumes in determining the biological activity. The other consists in stabilizing the drug in the conformation required for proper binding to the receptor. Both ways may be considered as static in that H-bonding acts along with other intermolecular forces in lowering the energy of the ligand–receptor complex. However, the dynamic properties of H-bonding might be involved in the second stage of receptor mechanisms, i.e. in the activation of enzymatic processes. We shall illustrate the first two ways by examples and the third by a model calculation.

HYDROGEN BONDING AND LIGAND CONFORMATION

The binding sites of enzymes and other ligand-binding proteins have a well-defined three-dimensional structure of the binding site into which the ligand must fit. There is a large body of evidence that this is also true of receptors. However, most of the natural ligands, and also synthetic drugs, are rather flexible molecules and this brings up the question of the so-called bioactive conformation, i.e. the one that best fits the binding site. For drug design it is de-
sirable that the molecule be possibly rigidified in the proper conformation in order to avoid entropy losses. However, lacking structural information on the binding site architecture, the bioactive conformation has yet to be worked out in an empirical way.

The quest for the bioactive conformation of ligands to the \( \text{H}_2 \)-histamine receptors presents itself with the natural agonist, histamine, as well as with the antagonists, used in anti-ulcer therapy. The side chain that connects various proton-donating groups to the imidazole ring might be extended or folded. Numerous theoretical studies (for references see ref. 8) were conducted to answer this question. Perhaps the best arguments in favour of the folded structure were presented by combining the synthesis of conformationally restricted antagonists with X-ray structure determinations and, obviously, activity studies [9,10]. The stabilization of the folded form (Fig. 1) is due to the intramolecular H-bond between the side-chain NH groups and the imidazole nitrogen as acceptor. This bond is shown in the X-ray structures of many \( \text{H}_2 \)-antagonists and also persists in solution [11,12].

For an interesting example of the role of H-bonding in conformational stability, but not in intermolecular bonding, the antitumor drug daunorubicin can be presented. This drug appears to intercalate DNA whereby the conformation of ring A and its 9-hydroxy substituent are important for activity (Fig. 2). X-Ray structure determination of its complex with a model hexanucleotide suggested that this group is involved in H-bonding to a guanine of the model DNA fragment. However, 9-deoxydaunorubicin binds to DNA with very similar affinity as daunorubicin itself but it is nearly inactive. Thus the role of the axially

![Fig. 1. Folded form of a cimetidine type \( \text{H}_2 \)-histaminic antagonist.](image)

![Fig. 2. Structure of 9-hydroxydaunorubicin.](image)
oriented 9-hydroxy group is not in intermolecular bonding to the receptor DNA, but in supporting the molecular conformation [13].

Instead of enumerating other examples of H-bonds stabilizing the bioactive conformation let us consider an opposite case, i.e. that breaking of an intramolecular H-bond is necessary for the drug to achieve proper conformation. This example may be considered as a warning against premature conclusions about the bioactive conformation. It concerns the conformation of the D ring of monoamides of lysergic acid which include the pharmacologically very important ergot alkaloids. The D ring of the ergolene skeleton may assume two low-energy half-chair conformations D₁ and D₂ (Fig. 3). Molecular mechanics calculations show the D₁ conformation to be more stable by about 6 kcal mol⁻¹ [14]. However, with primary lysergamides the conformation observed in NMR analysis [15] corresponds to the D₂ conformation and the same result is obtained by X-ray structure determinations [16]. The higher energy required for the D ring to assume this conformation is compensated by intramolecular H-bonding between the amide NH on C8 and N6 of the ergolene. This finding induced the conclusion that the D₂ conformation suits the receptor [17]. However, the spatial orientation of the electron lone pair of N6 is in this conformation at variance with the orientation of the N lone pair in other dopaminergic drugs with rigid conformations, apomorphine for instance [14].

The quandary was resolved by the NMR conformational analysis of some ergot alkaloids in DMSO solution and their salts in CDCl₃ solution. Under such circumstances the intramolecular H-bond does not exist and D₁ conformation prevails. Secondary lysergamides are not capable of forming the intramolecular H-bond and assume D₁ conformation [18]. The well-known psychotomimetic LSD exemplifies this. In this conformation the geometrical parameters characterizing the N lone pair are in good agreement with the ones calculated for conformationally restricted dopaminergic drugs [19].

INTERMOLECULAR H-BONDING

There is hardly any ligand molecule devoid of any group potentially acting as a proton donor or acceptor and usually there is more than just one such group. Each of the potentially H-bonding groups might contribute a certain amount to the interaction energy. Andrews et al. [20] have analyzed affinity data for 200 drugs and enzyme inhibitors in terms of contributions of various
functional groups and deduced average values which may be used in estimating $\Delta G$ of ligand binding to receptors. Thus a phenolic OH group may contribute 2.5 kcal mol$^{-1}$ and a charged nitrogen-containing group 11.5 kcal mol$^{-1}$. These are, of course, average values and, moreover, it has yet to be shown whether all groups present in a molecule are really engaged in bonding. From the latter point of view, the estimates of group contribution to the free energy of bonding, based on experiments with geometrically engineered enzymes are superior. Fersht [21] deduced from point mutations leading to replacement in tyrosyl t-RNA synthetase of, for example, a tyrosine by phenylalanine that individual uncharged H-bonds contribute 0.5–1.8 kcal mol$^{-1}$ to the free energy of binding, $\Delta G$, of inhibitors whereas charged bonds contribute 3–6 kcal mol$^{-1}$. These values appear low in comparison with values typical of dissociation energies determined in the gas phase or apolar solutions, but the values derived from the former type of evaluation include the energies of dehydration of both the donor and acceptor groups, and thus represent net contributions to $\Delta G$.

The case of enzyme inhibitor binding is clear cut since the engagement of, say, the tyrosine OH group is evident from X-ray structure determination. However, without such evidence, as is the case of pharmacological receptors, it is much more difficult to make certain that a potentially H-bonding group of the drug molecule really deploys the true H-bonding characteristics in binding to the receptor. Moreover, the maximum energy may not be achieved if steric constraints exist. Alternatively, a major component of H-bond energy might be involved, for instance the electrostatic, and this could be replaced by a corresponding contribution from another type of substituent.

In order to make it clear what is meant by involvement in binding of a component only of H-bonding energy, we shall consider agonists to serotonine receptors related to the physiologic one, 5-hydroxytryptamine. Tryptamine itself has a much lower affinity [22] and the same is true of 4- and, particularly, 7-hydroxytryptamines. This suggests that optimal receptor binding requires an OH group in the proper position. However, 5-amino- and 5-methoxy-tryptamine also display sizable affinities (Fig. 4). This might indicate that the 5-substituent should be a proton acceptor. However, 5-methyl as well as some other substituted tryptamines also bind to the serotonin receptor and thus affinity determinants with some other, more general physical character not connected with H-bonding have to be looked for. In fact, many substituent parameters, including charge transfer and topical electronic parameters, were attempted in quantitative structure–activity relationships (QSAR), but nothing satisfactory emerged [23]. Topographical characteristics of the molecular electrostatic potential appear to be physically most meaningful and were used in QSAR. However, with the best parameter, the averaged negative potential above the aromatic plane multiplied by the distance between the centre of gravity of the potential and the side-chain nitrogen, a correlation coefficient $r = 0.6$ was obtained [24]. This demonstrates the importance of electrostatic forces, but
obviously other factors must be involved in the interaction. These results suggested that a model approach to binding which permitted energy analysis might be helpful.

We have recently constructed [25] a simple, two-dimensional model of a receptor binding site (Fig. 5) inspired by the trp-aporepressor [26]. It consists of ethylguanidinium (curtailed side chain of arginine) H-bonded to formiate (curtailed side chain of glutamate or aspartate) and has no net charge but a strong dipole. Interaction energies with tryptamine and hydroxytryptamines were calculated with optimized geometries. In the optimization procedure, for which the AM1 method was adopted, the ligands were constrained to a plane parallel to the receptor model, thus mimicking the limited mobility of the ligand in a binding cavity. The energies were recalculated in the ab initio scheme with the minimal STO-3G basis set and decomposed according to the scheme of Kitaura and Morokuma [27]. The electrostatic component was dominant and the other components (polarization, exchange repulsion, charge transfer) followed in proportions similar to those obtained for H-bonded complexes [27]. The dispersion energy was calculated from empirical atom–atom potentials and it turned out to be even superior to the SCF interaction energy. This explained the failure of attempts to correlate purely electronic parameters with affinity, which was the actual purpose of the model calculations. The interaction energies were ranged 6-hydroxytryptamine < tryptamine < 5-hydroxytryptamine and corresponded to the rank order of affinities although no explicit bonding for the hydroxy substituents was provided in the model. The results appeared fortuitous and further calculations with different starting geometries showed that there are indeed several orientations of the ligand with respect to the model receptor that differ only slightly in energy.

The model considered so far is deficient with respect to the specificity of
Fig. 5. Tryptamine binding to the receptor model (ethylguanidinium-formiate): (a), (b), neutral tryptamine; (c) protonated tryptamine geometry optimized in the AM1 scheme.

There are two ways to improve on this. One is to extend the model by adding a binding site for the 5-substituent of the tryptamine ligand. Considering what was said above concerning the relation between the character of this substituent and the affinity, a hydroxyl group on the receptor, e.g. a serine side chain, would be a good choice. We did not include this in any calculation since it is easy to estimate that it would add at least 4 kcal mol$^{-1}$ to the interaction energy, thus fixing the ligand in a position dictated by the additional anchor on the receptor. The other thing to do is to take the ligand in the ionic form, i.e. with the nitrogen in the tryptamine side chain charged. There is strong evidence that, in fact, it is the ammonium form that preferably interacts with the receptor [28]. With the protonated ligand the interaction
TABLE 1

Interaction energies (kcal mol\(^{-1}\)) of a model receptor (ethylguanidinium-formiate) with tryptamine (AM1 calculation)

<table>
<thead>
<tr>
<th>Geometry (Fig. 5)</th>
<th>(E_{\text{int}}) (SCF)</th>
<th>(E_{\text{disp}}) *</th>
<th>(E_{\text{tot}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) neutral</td>
<td>-3.601</td>
<td>-11.64</td>
<td>-15.24</td>
</tr>
<tr>
<td>(b) neutral</td>
<td>-2.43</td>
<td>-4.36</td>
<td>-6.78</td>
</tr>
<tr>
<td>(a) protonated</td>
<td>-19.47</td>
<td>-11.73</td>
<td>-31.21</td>
</tr>
<tr>
<td>(b) protonated</td>
<td>+3.09</td>
<td>-4.36</td>
<td>-1.26</td>
</tr>
<tr>
<td>(c) protonated</td>
<td>-31.73</td>
<td>-7.48</td>
<td>-39.21</td>
</tr>
</tbody>
</table>


energy increases to about 30 kcal mol\(^{-1}\) and produces a large difference between the orientations shown in Fig. 5 and Table 1.

The geometry of the model receptor-ligand complex with the largest interaction energy contains an H-bond between the ammonium group of the ligand and formiate of the receptor. This is not only important for interaction energy but also permits conjecture about the mechanism of the second stage in receptor functioning, i.e. activation by agonists.

A POSSIBLE ROLE OF H-BONDING IN RECEPTOR ACTIVATION

The binding event has to be transmitted by the receptor protein to a distant site where the signal is picked up by the coupling protein which subsequently activates (or deactivates) biochemical processes regulated by the receptor. In essence the mechanism is related to allosterism in enzymes. Ligand binding can be considered as a perturbation in the protein force field that results in a conformational change. The perturbation may be inflicted by changes in particular linkages between amino acid side chains, for instance, salt bridges. Our receptor model, in fact, represents a rather common type of bridging between arginine and glutamate or aspartate side chains and we consider possible changes caused by the approaching charged ligand. For economy reasons, the latter is reduced to an ammonium ion. The ternary complex obtained by geometry optimization with the AM1 method [29] is shown in Fig. 6.

The ammonium ion placed at optimized H-bonding distance from formiate causes the latter to rotate after one of the guanidinium-formiate H-bonds has been disrupted. In optimizing the ternary complex (Fig. 6) all four protons were kept fixed to the ammonium nitrogen. On releasing this constraint one proton moved to formiate. The energy changes of these coupled processes are shown schematically in Fig. 7 and with more detailed energy functions in Fig. 8. The geometry optimizations were carried out by the semiempirical AM1
Fig. 6. Starting complex for the reactions in Fig. 7 (AM1 optimized).

Fig. 7. Energy scheme of changes in hydrogen bonding as a possible mechanism of receptor activation. Energies in kcal mol$^{-1}$ (AM1 scheme) and a.u. (in parentheses, ab initio calculation with 4-31G basis set).
Fig. 8. Energy functions for formiate rotation with breaking one guanidinium hydrogen bond and formation of a new bond to ammonium (a), proton transfer from ammonium to formiate (b), and back under the influence of a positive charge placed 1.5 Å above the oxygen (c).

method [21] and the energies recalculated in the ab initio scheme [30] with the 4-31G basis set. Although the energies of particular steps differ between the two methods, they are in the same direction and the final energy balance is not too different. However, the calculation was made "in vacuo" and it is well known that the proton potentials are very sensitive to medium effects [31], particularly to surrounding charges [32]. Thus a positive charge placed
1.5 Å above the guanidinium residue would send the proton in the formic acid-ammonia H-bond back to the nitrogen [Fig. 8(c)]. Thus the present model calculations show the feasibility of a proton transfer coupled to the rotation of a carboxylate group triggered by the approach of a charged ligand and may result in a conformational change of the protein.

Using a charged ligand for the receptor activation raises the question of the difference between agonists and antagonists. The latter most often carry the same type of aminic side chain as the agonists. This question also arises with another recently proposed model of serotonin receptor triggering [33]. The answer has to be sought in the different binding geometry of the two types of ligand and this must be connected with structural differences in the rest of the molecules. The argument of this hypothesis is lengthy and cannot be presented here. It is, however, much more questionable whether such rather specific mechanisms for triggering conformational changes of the receptor are needed at all. There is no direct or, at least, circumstantial evidence of proton transfer and the answer will have to await superior structural information on receptors permitting more realistic molecular modelling, such as is the case with enzymes. This will also establish more firmly the true role of particular H-bonds in the ligand–receptor interaction.

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