DOI: 10.1002/cmdc.200600251

Structure-Based Calculation of Binding Affinities of α_{2A}-Adrenoceptor Agonists

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Dedicated to Professor E. Sylvester Vizi on the occasion of his 70th birthday.

Adrenergic receptors of the α_2 type (α_2-adrenoceptors) belong to the family of seven transmembrane-spanning G-protein-linked receptors.[1–9] α_2-Adrenoceptors can be grouped into three highly homologous subtypes (α_{2A}, α_{2B}, and α_{2C}) and, because of the difference in pharmacology,[10] a fourth subtype (α_{2D}) can be formally distinguished, though this is rather a species orthologue.

In general, the α_2-adrenoceptors are responsible for the presynaptic feedback of the release of adrenaline and noradrenaline, their physiological agonists. Although numerous findings are available on the receptor subtypes from experiments with knockout mice[11] and these results are of some relevance for human pharmacology, the similar patterns of expression of adrenergic receptors in human and mouse tissues do not guarantee similar functions. Thus, the individual roles of the three α_2-adrenoceptor subtypes in humans have not been completely elucidated. However, the results of the reported studies do indicate (see Supporting Information) that the α_2-adrenoceptor subtypes are involved in various important physiological processes, and further investigations of the differences in their molecular pharmacology are therefore essential.

The identification of subtype-specific functions from pharmacological experiments is currently not possible because of the lack of subtype-specific ligands[3,6–8] and the cross-reactivity with imidazoline receptors.[7] The development of subtype-selective agonists would be useful as it would facilitate further examinations of the molecular pharmacology of the α_2-adrenoceptors. The rational, structure-based design of such agonists requires a precise knowledge of the molecular structure of the binding site. Unfortunately, because of the difficulties inherent in crystallization, atomic-resolution structures of the α_2-adrenoceptors are not available in the Protein Databank.

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Supporting information for this article is available on the WWW under http://www.chemmedchem.org or from the author.

In the present study, an atomic-resolution model of the α_{2A}-adrenoceptor was constructed through use of its amino acid sequence and the crystallographic bovine rhodopsin structure as a template. Similar homology models were earlier constructed by other researchers[12] and successfully used to provide qualitative explanations. The α_{2A}-adrenoceptor model in the present study is based on a crystallographic template structure with a resolution of 2.2 Å[13] appropriate for quantitative investigations (for details, refer to the Computational Methods below).

In possession of the atomic resolution target structure (α_{2A}-adrenoceptor), 15 known agonist ligands were automatically docked to the presumed binding region of the receptor (Figure 1a). Inspection of the results revealed that the docked ligand conformations are in physical contact with the key residues D3.32(113), S5.42(200), and S5.46(204), previously identified by site-directed mutagenesis studies.[14–16] As an example, the positively charged amino group of noradrenaline (Figure 1b) or of methylnoradrenaline forms a salt bridge with the negative side-chain carboxylate of D3.32(113). Similar results involving an interaction between the ionic groups were earlier obtained for noradrenaline.[13] For some other ligands (for example, clonidine, Figure 1c), interactions can be observed with E4.39(189) instead of D3.32(113) . Additionally, the binding pocket is formed by hydrophobic amino acids such as V5.39-(197), F5.47(205), W6.48(258), F6.49(259), F6.52(262), and the key serine residues.

The qualitative agreement with the site-directed mutagenesis data indicates the usefulness of the homology model and the docking procedure applied. However, a correct (quantitative) estimation of the binding free energy (ΔG_b) is the real challenge in molecular design. Once a ΔG_b calculator has been developed, the screening-out of potent (tight binding) agonists from the candidate compounds becomes possible. To meet this expectation, quantitative structure–activity relationships (QSARs) were developed by using the docked structures and experimental ΔG_b values of the agonists.

As a first attempt, simple linear regression (LR) was performed, involving the modified scoring function values (ΔG_T) of AutoDock 3.0 program package, which includes the intermolecular (enthalpic) terms and a solvation penalty. These values were calculated for the docked agonist–protein complex structures. A detailed discussion on the calculation of ΔG_T is to be found in Ref. [17]. An excellent correlation was obtained for nine ligands not containing chlorine atoms [Eq. (1), Figure 2].

$$\Delta G_b = \underset{\pm 0.1301}{\overset{t=12.3237}{1.6037}}\,\Delta G_T + \underset{\pm 1.0909}{\overset{t=3.9601}{4.3201}}$$
$$\left(r^2 = 0.96; r^2_{cv} = 0.93; F = 151.87; s^2 = 0.09; N = 9\right) \quad (1)$$

An inspection of the *t*-values indicates that both ΔG_T and the intercept are necessary parameters of the regression equation. The mean square errors of the regression coefficients, the *F* value, the standard deviation (s^2), the square of the correlation coefficient (r^2), and the leave-one-out cross-validated r^2 (r^2_{cv}) of the regressions reflect the statistical significance of the LR.

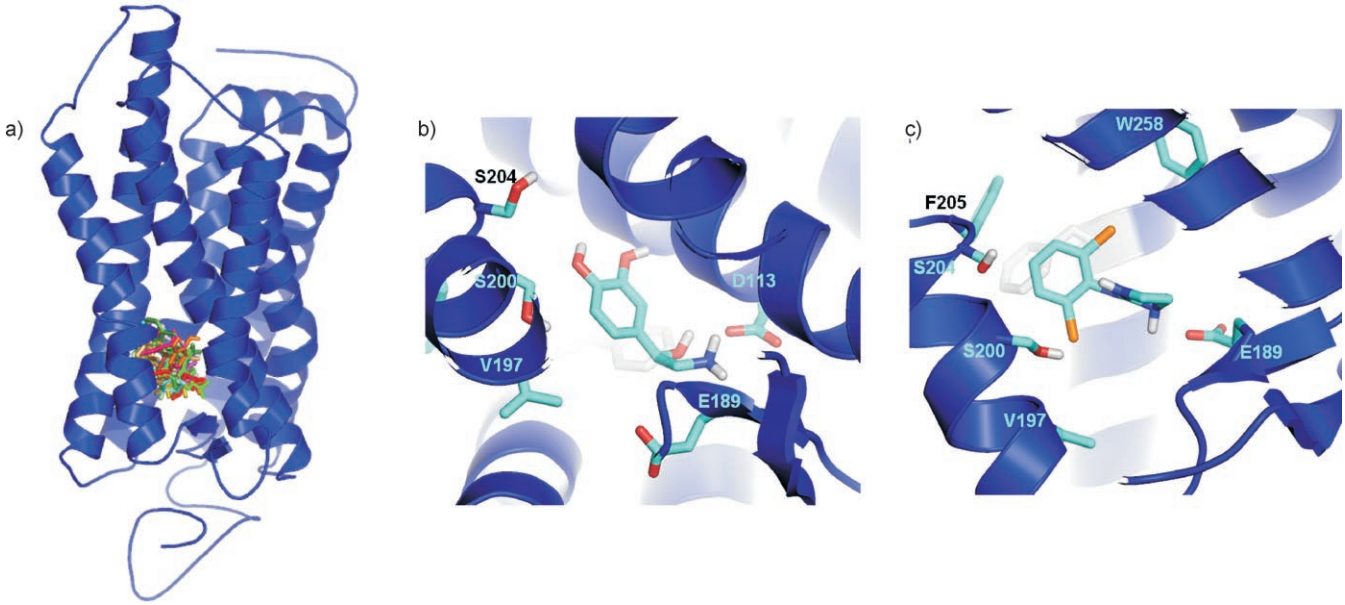


Figure 1. a) Structure of the homology modeled and energy minimized α_{2A}-adrenoceptor. Docked conformations of all 15 ligands are located in the same central binding cavity. b) Noradrenaline binding to the active site of the α_{2A}-adrenoceptor. Key residues of the site are denoted by sticks. A salt bridge is formed between the oppositely charged side-chain of D113 and the amino group of noradrenaline. c) Clonidine binding to the active site of the α_{2A}-adrenoceptor. Experimentally detected key residues of the site are denoted by sticks. A hydrophobic binding pocket is formed by W and F residues.

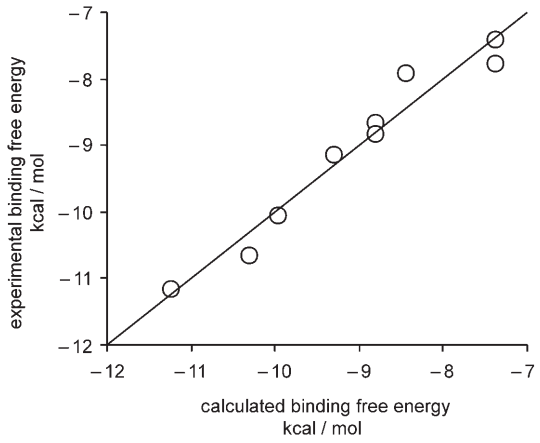


Figure 2. Correlation between the experimental and calculated binding free energies of nine agonists. Small residuals were obtained for nonchlorinated compounds with the use of only one descriptor: the modified AutoDock free energy function, ΔG_T [Eq. (1)].

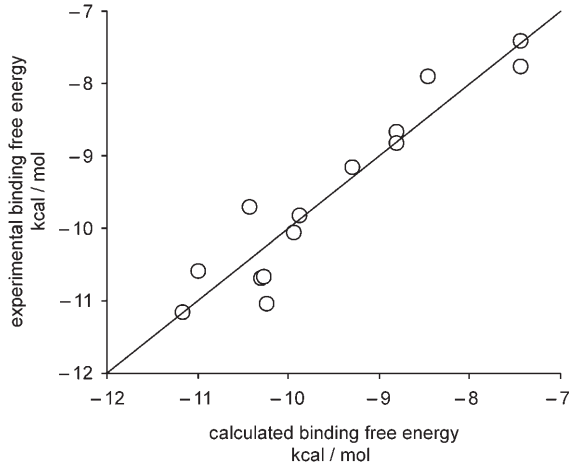


Figure 3. Correlation between the experimental and calculated binding free energies of all 14 agonists. Besides ΔG_T, involvement of a second descriptor resulted in a fair correlation for the chlorinated compounds too [Eq. (2)].

Five chlorinated agonists do not satisfy Equation (1). The common feature of these five molecules is that all of them have a 2,6-dichloro substituted phenyl (2,6-DCP) ring. Thus, it is plausible to involve a binary descriptor of existence (E) of the 2,6-DCP ring in the regression, which accounts for the presence or absence of this moiety, that is, $E=1$ (or 0) if there is (or is not) a 2,6-DCP ring in the ligand. Inclusion of this descriptor yields a three-parameter LR [Eq. (2), Figure 3]:

$$\Delta G_b = \underset{\pm 0.1875}{\overset{t=8.2915}{1.5543}}\,\Delta G_T - \underset{\pm 0.2524}{\overset{t=-7.3038}{1.8434}}\,E + \underset{\pm 1.5718}{\overset{t=2.4860}{3.9075}}$$

$$\left(r^2 = 0.90; r_{cv}^2 = 0.84; F = 48.62; s^2 = 0.19; N = 14\right) \quad (2)$$

Similarly as for Equation (1), this multiple LR is statistically relevant and only one (dexmedetomidine) of the 15 agonists was an outlier with a residual > 1.5 kcal mol^{-1}, and had to be omitted from the final LR. ΔG_T includes mostly intermolecular (enthalpic) contributions to ΔG_b[17] and the constants 4.3201 and 3.9075 kcal mol^{-1} in Equations (1) and (2), respectively, sufficiently represent the entropic loss due to freezing of translational, rotational, and torsional degrees of freedom in the nine ligands. However, descriptor E in Equation (2) requires further discussion. Notably, the sign of the coefficient of E is negative. This means that the presence of a 2,6-DCP ring is favorable for binding, indicating two possibilities. 1) The substituent chlorine

atoms are involved in interactions with the protein which are not correctly represented by ΔG_T. Comparison of the atomic contributions of the chlorine to the electrostatic and van der Waals terms of ΔG_T with those of other ligand atoms with a similar character (for example, oxygen) allows the conclusion that the enthalpic contributions are not underrepresented for the chlorine atoms. 2) The presence of the 2,6-DCP ring alters the entropy of binding. Conformational energy diagrams for the phenyl rotation (Supporting Information) show that the energy gap between the stable and the high energy conformation is twice as high for the 2,6-DCP ring as it is for the simple phenyl ring. Besides this intramolecular interaction effect, the heavy chlorine atom may alter the corresponding rotational frequency too. Certainly, movements of the phenyl rotor are restricted following chlorine substitution, and the entropic loss of this freezing rotor is therefore smaller. This decrease in the entropic loss may be a realistic explanation of the negative sign of E in Equation (2). Involvement of other 2,6-DCP ring-containing (at any event not a *para*-chlorophenyl-containing) ligand–protein complexes and quantum chemical calculations would be necessary for a detailed elucidation, but that is beyond the scope of the present study. The structures and conformational degrees of freedom of the ligand molecules within the two, that is, chlorinated and nonchlorinated subsets are similar. Thus, our results agree with the rational assumption that the binding entropy is approximately the same for the ligands within the two subsets.

The experimental ΔG_b values of these 15 agonists were converted from the pK_i (logarithm of inhibition constant) values obtained from radioligand assays. For nine of the 15 compounds, the pK_i values were determined with two different radioligands [^{3}H] MK-912 and [^{3}H] RX821002. A LR using the corresponding two vectors of the experimental ΔG_b data yields valuable information on the interchangeability and reproducibility of the available experimental data. Although the two vectors are correlated ($r^2 = 0.76$) with each other, the statistical parameters (see the Supporting Information for details) of this correlation are not as fascinating as might be hoped. Thus, in the present study, it was a good choice to use experimental data obtained with only one radioligand ([^{3}H] MK-912) for QSAR building.

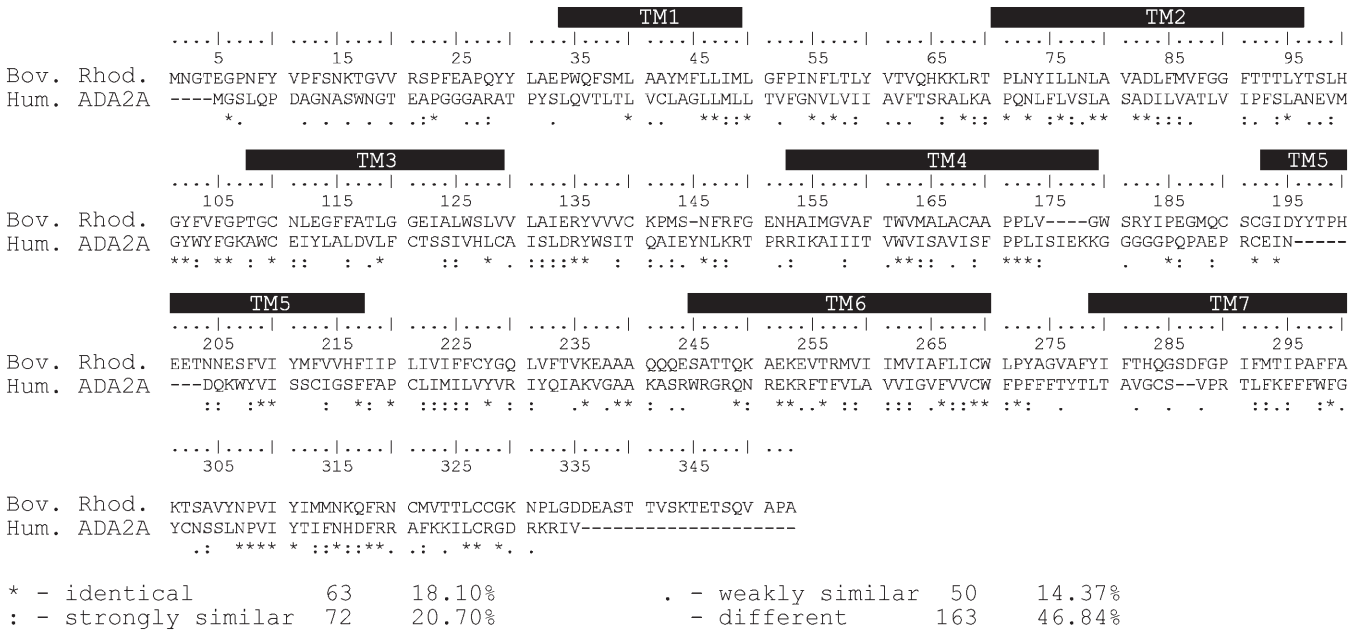
In conclusion, 15 agonists with various structures were docked to an atomic resolution homology model of the human α_{2A}-adrenoceptor. The docked conformations of the compounds are in contact with previously reported key binding site residues emphasizing the good quality of the homology model. QSARs of binding affinity were developed involving structure-based bimolecular terms of the AutoDock scoring function, a simple, ligand-based binary descriptor, and a set of the corresponding experimental ΔG_b values. A good correlation was achieved between the experimental and calculated ΔG_b values. The statistical parameters of the LRs are somewhat better than those of the reproducibility of the experimental data. To the best of our knowledge, this study represents the first verified calculations of binding affinities of agonists to the α_{2A}-adrenoceptor. Thus, our results indicate the direction of precise engineering of agonists, either for the elucidation of open questions of subtype selectivity (see introductory sections) or for the design of drug candidates in α_{2A}-adrenoceptor-related diseases and therapeutic issues such as hypertension,[18–19] glaucoma,[20] acute migraine,[21] analgesia, anesthesia, sedation,[22–24] drug and alcohol withdrawal,[25–27] gastroprotective effects,[28–30] and Parkinson's disease.[31–32]

Computational Methods

Homology modeling and refinement. The amino acid sequences of both bovine rhodopsin (template protein) and the human α_{2A}-adrenoceptor were obtained from the online protein database.[33] Both sequences were loaded in Bioedit 7.0.5.2[34] and were aligned (Figure 4) with ClustalW 1.4. Manual correction of the alignment was performed if needed (see Supporting Information). A bovine rhodopsin coordinate file (Protein Databank code: 1U19) of 2.20 Å[13] was selected as the structural template. Modeller 8v1[35–37] was used for model building. Inputs of Modeller were the protein coordinates of 1U19, the amino acid sequences, and a file containing the options of the calculations. One hundred α_{2A}-adrenoceptor homology models were created and the model with the lowest modeller objective function value was selected. The quality of the model was checked with the web version of the program ProCheck1.5[38–39] (see Supporting Information). The α_{2A}-adrenoceptor homology model with a blind docked[40] noradrenaline ligand conformation sitting at the binding region was refined by GROMACS[41] molecular mechanics minimization, as described previously.[17]

Docking and scoring. The structures of the 15 agonist molecules (noradrenaline, α-methyl-noradrenaline, B-HT 920, brimonidine, clonidine, dexmedetomidine, guanabenzamidine, guanfacine, levlofexidine, oxymetazoline, *p*-aminoclonidine, rilmenidine, st91, xylometazoline, and a54741) were built, optimized, and supplied with Gasteiger charges by using the SYBYL program package and force field.[42–44] The docking box with $22.5 \times 22.5 \times 22.5$ Å^3 volume was centered at the binding region known from site-directed mutagenesis studies.[6] All docking calculations were performed as in Ref. [45], using the AutoDock 3.0 program package.[46] Ligand molecules with different proton locations were investigated in cases where protonation was not trivial. Protonated forms (and the corresponding ΔG_T) resulting in the smallest residuals were selected for QSAR. Detailed results of docking are tabulated in the Supporting Information. Molecular graphics was prepared with PyMol.[47]

Linear regressions. QSARs were developed with the CODESSA program package[48–50] and its two-dimensional descriptor pool. The binary descriptor E was constructed manually, and included in the pool and selected automatically by the improve correlation module of CODESSA. Experimental ΔG_b values used in the LRs (Supporting Information) were converted from pK_i values ($T = 298$ K) of previous studies of radioligand assays.[51–53] Only pK_i values obtained with radioligand [^{3}H] MK-912 were used to construct Equations (1) and (2). When more than one experimental pK_i value was available, the larger one was selected for correlation.



* - identical 63 18.10% . - weakly similar 50 14.37%
: - strongly similar 72 20.70% - different 163 46.84%

Figure 4. The sequence alignment of bovine rhodopsine (template) and human α_{2A} adrenoceptor (target) proteins. There is approximatately 40% similarity (identical + strongly similar) between the sequences of the two proteins. Transmembrane regions are marked with TM.

Acknowledgements

We thank the National Office for Research and Technology (NKTH), Hungary for support of this study. C.H. is greatful to Foundation Innove (www.innove.ee) project No. 1.0101-0310 for financial support, and the referee for useful suggestions on the manuscript.

Keywords: alpha$_{2a}$-adrenerg receptor · docking · free energy · homology modeling · QSAR

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Received: October 25, 2006
Revised: February 5, 2007
Published online on April 16, 2007

