Molecular modelling of the human A2b adenosine receptor and an analysis of the binding modes of its selective ligands

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The first molecular model of the human A2b adenosine receptor has been created, and the binding modes of its selective ligands have been studied.

The P1 receptor family comprises A1, A2a, A2b, and A3 adenosine receptors, which were identified by convergent data from molecular, biochemical and pharmacological studies. All of them are G-protein coupled receptors (GPCRs).[1] Like other GPCRs, adenosine receptors have a central common core composed of seven transmembrane helices (TM-I to TM-VII) connected by three intracellular and three extracellular loops. Adenosine receptors are widely distributed in most species and mediate diverse biological effects. Because of this, the ligands of these receptors are widely used in pharmacology and medicine.[2] Despite intensive efforts in this area, there are no A2b-selective agonists. Adenosine receptors, like the other GPCRs, are integral membrane proteins. Such macromolecules are not easily amenable to crystallization and, hence, to precise structure elucidation through X-ray diffraction. For this reason, molecular modelling is the most applicable method for the determination of the structure of GPCRs. However, no molecular models of the A2b subtype of adenosine receptors were reported in the literature.

Here, we describe the first molecular model of the A2b adenosine receptor and the binding of its ligands.

A sequence alignment of four subtypes of the adenosine receptors and rhodopsin[3] was constructed to determine amino acids, which form a transmembrane alpha-helical domain (Scheme 1).

The primary sequences were taken from the SWISSPROT protein data bank.[4] Then, the amino acids of rhodopsin were replaced by the amino acids of the A2b receptor using the COMPOSER block of Sybyl 6.7.2.[5] The created model of the A2b receptors was optimised by molecular mechanics methods using the Tripos force field.[5] Atomic charges were retrieved from the KOLLMAN-ALL dictionary.[5] Then, the extracellular and intracellular hydrophilic loops were inserted into the model using the LOOP SEARCH command of the Sybyl 6.7.2 package.[5]

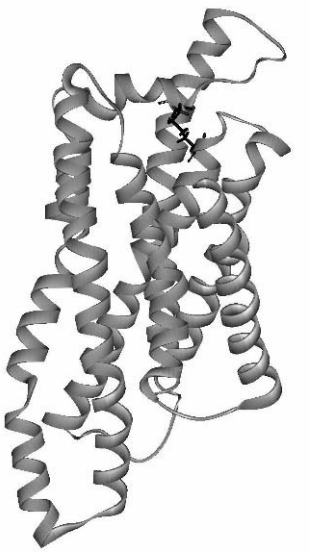

Figure 1 Model of the human A2b adenosine receptor.

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TM1
1  10  AAYIGIEVLIALVSVPGNVLVIWAVK  35
2   7  SVYITVELAIAVLAILGNVLVCWAVW  32
3   8  ALYVALELVIAALSVAGNVLVCAAVG  33
4  13  VTYITMEIFIGLCAIVGNVLVICVVK  38
5  38  SMLAAYMFLLIMLGFPINFLTLYVTV  63

TM2
1  42  DATFCFIVSLAVADVAVGALVIPLAI  67
2  39  NVTNYFVVSLAAADIAVGVLAIPFAI  64
3  40  TPTNYFLVSLAAADVAVGLFAIPFAI  65
4  45  TTTFYFIVSLALADIAVGVLVMPLAI  70
5  70  TPLNYILLNLAVADLFMVFGGFTTTL  95

TM3
1   81  LMVACPVLILTQSSILALLAIAVDRY  106
2   78  LFIACFVLVLTQSSIFSLLAIAIDRY  103
3   79  LFLACFVLVLTQSSIFSLLAVAVDRY  104
4   84  LFMTCLLLIFTHASIMSLLAIAVDRY  109
5  111  NLEGFFATLGGEIALWSLVVLAIERY  136

TM4
1  122  RRAAVAIAGCWILSFVVGLTPMF  144
2  119  TRAKGIIAICWVLSFAIGLTPML  141
3  120  TRARGVIAVLWVLAFGIGLTPFL  142
4  125  RRIWLALGLCWLVSFLVGLTPMF  147
5  151  NHAIMGVAFTWVMALACAAPPLV  173

TM5
1  179  YMVYFNFFVWVLPPLLLMVLIYLEVF  204
2  176  YMVYFNFFACVLVPLLLMLGVYLRIF  201
3  181  YMVYFNFFGCVLPPLLIMLVIYIKIF  206
4  176  YMVYFSFLTWIFIPLVVMCAIYLDIF  201
5  202  SFVIYMFVVHFIIPLIVIFFCYGQLV  227

TM6
1  232  IAKSLALILFLFALSWLPLHILNCIT  257
2  231  AAKSLAIIVGLFALCWLPLHIINCFT  256
3  232  AAKSLAMIVGIFALCWLPVHAVNCVT  257
4  228  TAKSLFLVLFLFALSWLPLSIINCII  253
5  250  VTRMVIIMVIAFLICWLPYAGVAFYI  275

TM7
1  268  ILTYIAIFLTHGNSAMNPIVYAFRIQ  293
2  268  WLMYLAIVLSHTNSVVNPFIYAYRIR  293
3  270  WAMNMAILLSHANSVVNPIVYAYRNR  295
4  262  LVLYMGILLSHANSMMNPIVYAYKIK  287
5  286  IFMTIPAFFAKTSAVYNPVIYIMMNK  311
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(1 is A1; 2 is A2a; 3 is A2b; 4 is A3; 5 is rhodopsin, and TM is a transmembrane domain).

Scheme 1

Each loop was built from a template selected from a local Sybyl database containing the experimental geometry of the main chains of protein loops. The geometry of the created model containing all trans-membrane alpha-helices and hydrophilic loops was optimised (Figure 1). Two cystein residues located in the first and second extracellular loops appeared to be arranged at a distance suitable for the formation of the disulfide bond characteristic for all GPCRs belonging to family 1.[6] The geometrical parameters of the created model were checked using the PROCHECK program.[7] The quality of the model was tested by performing the molecular docking of adenosine as the nonselective native agonist of the adenosine receptor. The docking of adenosine and other

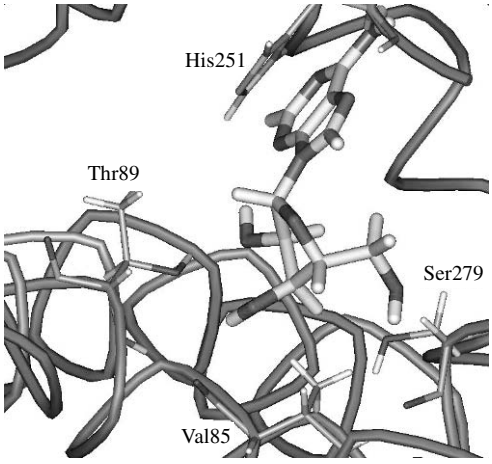

Figure 2 Binding mode of adenosine.

ligands was carried out manually using the DOCK command of Sybyl 6.7.2.[5] The geometry of the adenosine–protein complex was optimised using molecular mechanics methods and the Tripos force field.[5] Although structural differences between active and inactive states of the receptor (typical of protein complexes with an agonist and an antagonist, respectively[6]) cannot be explored using molecular mechanics, this approach is commonly used for studying ligand–receptor interactions inside the binding site of a protein.

The results on the docking of adenosine are in good agreement with published data for other subtypes of the adenosine receptors.[8,9] In general, the binding mode of adenosine with the A2b receptor is analogous to that of the binding to the A2a subtype:[9] the 5′-OH group interacts with Ser279 and Thr89 interacts with 2′- and 3′-hydroxyl groups (Figure 2). Additionally, His251 and Val85 bind with the ligand through hydrogen bonds with the nitrogen atom N7 and the 5′-OH group, respectively.

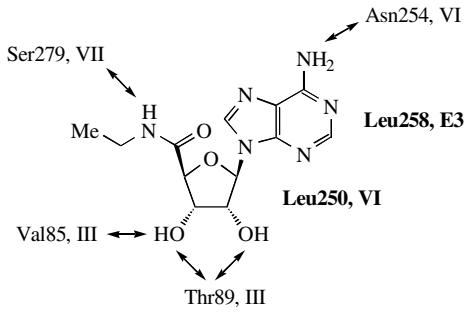

Figure 3 Binding mode of NECA.

Next, the most potent (although nonselective) agonist of the A2b subtype *N*-ethylcarboxamidoadenosine (NECA)[10] was docked to the receptor model. The results suggest that the hydroxyl group of Ser279 forms a hydrogen bond with the NH group at the 5′-position, while the carbonyl group of Asn254 interacts with the N6-amino group of the ligand. Furthermore, Val85 forms a hydrogen bond with the 3′-hydroxyl group, while Thr89 interacts with both 3′ and 2′-hydroxyl groups of the ligand (Figure 3). This result is in good agreement with the docking mode of adenosine and with the published data[9] for the A2a receptor. Val250, which is a nonconservative residue located at TM-VI, and Leu258, which is a partially nonconservative residue located at the third extracellular loop, are arranged in the immediate proximity of NECA.

The results of the docking of the most potent A2b antagonist 1,3-dipropyl-8-sulfophenylxanthine (DPSPX)[11] suggest that three amino acids of the receptor interact with the antagonist: Ser92, His251, and Asn239 (Figure 4). Ser92 and Asn239 form hydrogen bonds with a carbonyl group at the 2-position of the xanthine ring, while His251 interacts with an oxygen atom of the sulfo group. There are no nonconservative amino acids within

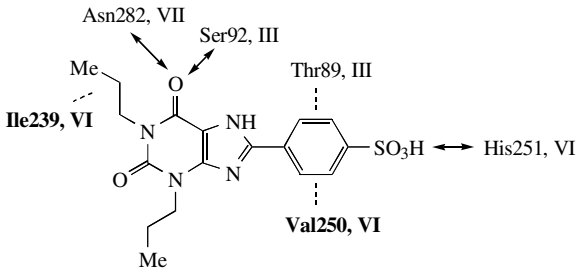

Figure 4 Binding mode of DPSPX.

a radius of 3 Å around the DPSPX. However, there are two nonconservative residues (Ile239 and Val250) within a radius of 4 Å around the ligand. The benzene ring of the ligand is located inside the hydrophobic pocket formed by Thr89, His251 and Val250.

Thus, the first model of the A2b adenosine receptor containing seven trans-membrane alpha-helices and all hydrophilic loops has been created. The docking of the most potent ligands and the native agonist of the A2b receptors have been performed using this model. The binding modes of the agonists and antagonists of the A2b receptors have been studied, and amino acids important for ligand binding have been identified. We assume that nonconservative amino acids Ile239 and Val250, which are located near the ligand, should be most important for the binding of selective ligands. In our view, new more selective ligands of the A2b receptor should be designed so that the interaction with these important amino acids should increase.

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