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3D HOMOLOGY MODEL OF THE alpha2A ADRENERGIC RECEPTOR SUBTYPE

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Modeling of the three-dimensional structure of the human alpha2A adrenergic receptor (alpha2A-AR) subtype based on the high-resolution X-ray structure of the human beta2-AR (2RH1, PDB file) is presented. The sequence of the alpha2A-AR subtype was aligned with the sequence of the 2RH1 template and the 3D homology model of the alpha2A-AR was built using the Swiss-PDBViewer software. The validation of the 3D homology model was performed by using PROCHECK software and by docking the endogen ligand, norepinephrine.

INTRODUCTION

Due to their high pharmaceutical importance, GDP binding (G) protein-coupled receptors GPCRs are attractive targets for drug-design programs. Generally, this requires knowledge of a three-dimensional structure of the receptor. However, given their location (transmembrane (TM) proteins) and other factors such as low natural abundance and difficulty in obtaining significant amounts of pure and active recombinant proteins, the attempts to obtain X-ray structures were very difficult and mostly unsuccessful. Therefore, modeling techniques have been employed to obtain insights in the three-dimensional structure of GPCRs. In 1993 and 1997 Baldwin analyzing around 200 and 500 GPCR sequences respectively, obtained a schematic overall structure establishing the probable arrangement of the seven TMs.[1,2] These 3D models proved useful for guiding experimental studies such as site-directed mutagenesis. In 2000, Palczewski et al. succeeded in solving a high-resolution X-ray structure of bovine rhodopsin as first example of an experimentally determined high-resolution three-dimensional structure of a GPCR.[3] The 3D structure of bovine rhodopsin in complex with its covalently bound inverse retinal agonist was very soon obtained at 2.2 Å.[4] More recently the X-ray spectrum at 2.4 Å resolution was published for carazolol-bound engineered beta2-AR fusion protein with T4 lysozyme (T4L) in which T4L replaces most of the third intracellular loop of the GPCR.[5] Last, the X-ray structure at 2.8 Å resolution of an engineered human beta2-AR-T4L chimera in complex with cholesterol has been published [3D4S].[6] Despite the missing of the third intracellular loop, the beta2-AR-T4L chimera protein was shown to retain near-native pharmacologic properties of beta2-AR. This high-resolution structure provides insights into ligand binding and the structural changes required to accommodate catecholamine agonists. The experimental-determined geometry of the human beta2-AR is today the best template[7] for building other 3D models of GPCR receptors, with unknown experimental 3D structure, especially for those from the rhodopsin-like family such as the three subtypes alpha2A-, alpha2B-, and alpha2C-AR of alpha2-ARs. These are important targets for the therapy of heart failure,[8, 9] hypertension,[10,11] attention deficit hyperactivity disorder,[12,13] glaucoma.[14] Although the three alpha2-AR subtypes have important cardiovascular, respiratory and neurological effects, they have not yet selective agonists, which can distinguish among the subtypes. Therefore, 3D models of these receptors can help to gain insight into their subtype specificity. *De novo* 3D models of the beta2-AR and alpha2-AR subtypes were published

by Kontoyianni *et al.*[15] In 1999 Johnson *et al.*[16] demonstrated the superiority of the 3D model of an alpha2A-AR based on the rhodopsin template[2] compared to a model based on the X-ray structure of bacteriorhodopsin. Combining theoretical and experimental methods Johnson et al. defined a scheme for catecholamine agonist[17,18] and antagonist[19] binding. All these models are based on rhodopsin templates. In this paper we report building, refinement and geometrical characteristics of a 3D-model of alpha2A-AR based on a human beta-AR template.

METHODS

Homology modeling of the human alpha2-AR was performed using as template the X-ray structure at 2.4 Å resolution of the human beta2-AR, PDB code file 2RH1.[6] The sequences of the human alpha2A and beta2-AR were extracted from the SwissProt database.[20]

The two sequences were aligned using the T-Coffee[21] software and the resulted alignment was submitted for automatic model building to the Swiss-PdbViewer[22-24] server.

The resulted model was then evaluated using the PROCHECK[25,26] program. Refinement of the model was performed with the HyperChem7.52 software.[27]

RESULTS

Sequences alignment of the alpha2A-AR, beta2-AR and 2RH1 template showed that in 2RH1, the 3D structure of the beta2-AR, the following fragments were missing: Asn1- Arg28, Gln231 – Ser262 and Arg343 – Leu413. The sequence Pro230 – Arg363 from the third intracellular loop of the alpha2A-AR has no correspondent sequence in the 2RH1 template, but this does not influence the binding site located in the transmembranar region. Therefore the third intracellular loop of the alpha2A-AR was deleted from Arg228 to Gly364. In Table 1 is shown the alignment before the deletion of the amino acids from the third intracellular loop.

In the final alignment the presence of highly conserved residues was checked. The following conserved amino acids (marked with bold characters in Table 1) of the alpha2A-AR can be identified: on the TM1 Gly1.49, Asn1.50 and Val1.53, on TM2 Ser2.45, Leu2.46, Ala2.47, Ala2.49 and Asp2.50, on TM3 Ser3.40, Leu3.44, Ile3.47, Ser3.48, Asp3.49, Arg3.50, Tyr3.51, Ile3.54, on TM4 Trp4.50, Ser4.53 and Pro4.60, on TM5 Phe5.47, Pro5.50, Ile5.53, Tyr5.58 and Ile5.61 (Val5.61 on 2RH1), on TM6 Arg6.32 (Lys6.32 for 2RH1) Phe6.44, Cys6.47, Trp6.48, Pro6.50, on TM7 Asn7.45, Ser7.46, Asn7.49, Pro7.50, Tyr7.53, Phe7.60 and Arg7.61 The amino acids have been numbered according to the Ballesteros-Weinstein convention.[28] In this convention the most conserved amino acid on a certain transmembranar helix is noted with 50 preceded by the number of the transmembrane helix. For example, in the third transmembranae helix the most conserved amino acid is an Arg noted 3.50. The rest of the amino acids on this helix are numbered starting from Arg3.50. For example the precedent amino acid is Asp3.49 and the following amino acid after Arg3.50 is a Tyr3.51. This sequence Asp-Arg-Tyr (or DRY in one letter notation of amino acids) is one of the most conserved sequences in the GPCR superfamily. On helix 3, Asp113 (Asp3.32) was shown to be implicated in interaction with alpha2A-AR agonists.

The model building was carried out using the Swiss-PdbViewer program[22-24] by generating a *framework* from the highly conserved residues (bold letters in table 1), which in the next step is filled up by loop building. Finally, side chain coordinates are generated. The loops having the same length as the corresponding domains in the template (2RH1) were created generating the atomic coordinates of the 2RH1 backbone. For the other cases a search routine of Swiss-PdbViewer program based on homology criteria was applied.

The validation step was performed using the PROCHECK software which verifies the normality of torsion angles, bond angles, bond lengths and distances between unbounded neighbor atoms.

A good 3D model should have over 90% residues in the most favored regions[16,17] of the Ramachandran plot.[29] The initial 3D model of the alpha2A-AR was refined in many steps. To avoid close contacts and to correct some distorted bond lengths and angles or planarity of some aromatic rings signaled by PROCHECK the geometrical parameters of the amino acid residues have been optimized by using the AMBER99 force field from HyperChem7.52. The refined 3D model contained 243 residues in the most favored regions (96.8 % in A, B ,L regions of the Ramachandran plot), 8 in the additional allowed regions (3.2% in a, b ,l, p regions) and none in each of generously allowed (~a, ~b, ~p, ~l regions) or disallowed regions (white area). The Ramachandran map for the refined 3D model of the alpha2A-AR based on 2RH1 template is displayed in Fig. 1.

Table 1

Sequences of human alpha2A-AR (SwissProt ID ADA2A_HUMAN, P08913) and beta2-AR (PDB file 2rh1) aligned with T_Coffee software

hA2A	-	-	-	-	-	-	-	M	G	S	L	Q	P	D	A	G	N	A	-	-	-	S	W	N	G
2RH1	D	Y	K	D	D	D	A	M	G	-	-	Q	P	G	N	G	S	A	F	L	L	A	P	N	R
								*	*			*	*	.		*	.	*				:		*	
hA2A	T	E	A	P	G	G	G	A	R	A	T	P	Y	S	L	Q	V	T	L	T	L	V	C	L	A
2RH1	S	H	A	P	D	H	D	V	T	Q	Q	R	D	E	V	W	V	-	V	G	M	G	I	V	M
	:	.	*	*	.		.	.						.	:		*		:		:			:	
hA2A	G	L	L	M	L	L	T	V	F	**G**	**N**	V	L	**V**	I	I	A	V	F	T	S	R	A	L	K
2RH1	S	L	I	V	L	A	I	V	F	**G**	**N**	V	L	**V**	I	T	A	I	A	K	F	E	R	L	Q
	.	*	:	:	*		*	*	*	*	*	*	*	*	*		*	:		.		.		*	:
hA2A	A	P	Q	N	L	F	L	V	**S**	**L**	**A**	S	**A**	**D**	I	L	V	A	T	L	V	I	P	F	S
2RH1	T	V	T	N	Y	F	I	T	**S**	**L**	**A**	C	**A**	**D**	L	V	M	G	L	A	V	V	P	F	G
	:			*		*	:	.	*	*	*	.	*	*	:	:	:	.			*	:	*	*	.
hA2A	L	A	N	E	V	M	G	Y	W	Y	F	G	K	A	W	C	E	I	Y	L	A	L	**D**	V	L
2RH1	A	A	H	I	L	M	K	M	W	T	F	G	N	F	W	C	E	F	W	T	S	I	**D**	V	L
		*	:		:	*			*		*	*	:		*	*	*	:	:		:	:	*	*	*
hA2A	F	C	T	S	**S**	I	V	H	**L**	C	A	**I**	**S**	L	**D**	**R**	**Y**	W	S	**I**	T	Q	A	I	E
2RH1	C	V	T	A	**S**	I	E	T	**L**	C	V	**I**	**A**	V	**D**	**R**	**Y**	F	A	**I**	T	S	P	F	K
			*	:	*	*			*	*	.	*	:	:	*	*	*	:	:	*	*	.	.	:	:
hA2A	Y	N	L	K	R	T	P	R	R	I	K	A	I	I	I	T	V	**W**	V	I	**S**	A	V	I	S
2RH1	Y	Q	S	L	L	T	K	N	K	A	R	V	I	I	L	M	V	**W**	I	V	**S**	G	L	T	S
	*	:				*		.	:		:	.	*	*	:		*	*	:	:	*	.	:		*
hA2A	F	P	**P**	L	I	S	I	E	K	K	G	G	-	-	-	G	G	G	P	Q	P	A	E	P	R
2RH1	F	L	**P**	I	Q	M	H	W	Y	R	A	T	H	Q	E	A	I	N	C	Y	A	E	E	T	C
	*		*	:					:	.						.		.			.		*	.	
hA2A	C	E	I	N	D	Q	K	W	Y	V	I	S	S	C	I	G	S	**F**	F	A	**P**	C	L	**I**	**M**
2RH1	C	D	F	F	T	N	Q	A	Y	A	I	A	S	S	I	V	S	**F**	Y	V	**P**	L	V	**I**	**M**
	*	:	:			:	:		*	.	*	:	*	.	*		*	*	:	.	*		:	*	*
hA2A	I	L	V	**Y**	V	R	**I**	Y	Q	I	A	K	R	R	T	R	V	P	P	S	R	R	G	P	D
2RH1	V	F	V	**Y**	S	R	**V**	F	Q	E	A	K	R	Q	L	K	F	-	-	-	-	-	-	-	-
	:	:	*	*		*	:	:	*		*	*	*	:		:	.								
hA2A	A	V	A	A	P	P	G	G	T	E	R	R	P	N	G	L	G	P	E	R	S	A	G	P	G
2RH1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
hA2A	G	A	E	A	E	P	L	P	T	Q	L	N	G	A	P	G	E	P	A	P	A	G	P	R	D
2RH1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
hA2A	T	D	A	L	D	L	E	E	S	S	S	S	D	H	A	E	R	P	P	G	P	R	R	P	E
2RH1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
hA2A	R	G	P	R	G	K	G	K	A	R	A	S	Q	V	K	P	G	D	S	L	P	R	R	G	P
2RH1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
hA2A	G	A	T	G	I	G	T	P	A	A	G	P	G	E	E	R	V	G	A	A	K	A	S	R	W
2RH1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
hA2A	R	G	R	Q	N	R	E	K	**R**	F	T	F	V	L	A	V	V	I	G	V	**F**	V	V	**C**	**W**
2RH1	-	K	F	C	L	K	E	H	**K**	A	L	K	T	L	G	I	I	M	G	T	**F**	T	L	**C**	**W**
						:	*	:	:				.	*	.	:	:	:	*	.	*	.	:	*	*
hA2A	F	**P**	F	F	F	T	Y	T	L	T	A	V	G	C	S	V	P	R	-	T	L	F	K	F	F
2RH1	L	**P**	F	F	I	V	N	I	V	H	V	I	Q	D	N	L	I	R	K	E	V	Y	I	L	L
	:	*	*	*	:	.			:		.	:			.	:			:		:	:		:	:
hA2A	F	W	F	G	Y	C	**N**	**S**	S	L	**N**	**P**	V	I	**Y**	T	I	F	N	H	D	**F**	**R**	R	A
2RH1	N	W	I	G	Y	V	**N**	**S**	G	F	**N**	**P**	L	I	**Y**	-	C	R	S	P	D	**F**	**R**	I	A
		*	:	*	*		*	*	.	:	*	*	:	*	*				.		*	*	*		*
hA2A	F	K	K	I	L	C	-	-	R	G	D	R	K	R	I	-	-	-	-	-	-	-	-	-	-
2RH1	F	Q	E	L	L	C	L	R	R	S	S	L	K	A	Y	G	N	G	Y	S	S	N	G	N	T
	*	:	:	:	*	*			*	.	.		*												
hA2A	-	-	-	-	V																				
2RH1	G	E	Q	S	G																				

amino acid one letter notation is used; **"*"** - identical amino acids in both sequences; "**:**" - amino acids highly similar; "**.** " - amino acids with low similarity; blank - dissimilar amino acids; bold amino acids - highly conserved amino acids in the GPCR family

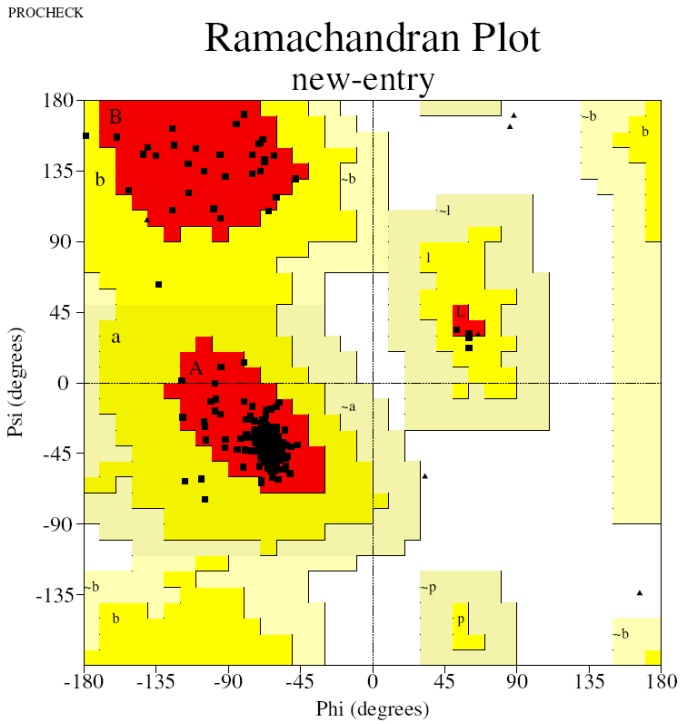

Fig. 1 – Ramachandran map for Alpha2A-AR after refinement steps.

Using the PROCHECK software the main-chain parameters (peptide bond planarity, bad non-bonded interactions, Cα distortion, overall G-factor), main-chain bond length distributions, main-chain bond angle distributions, side-chain parameters, etc. were also verified. All are in the admitted error limits or better. The 3D model, represented as a solid ribbon is displayed in Fig. 2.

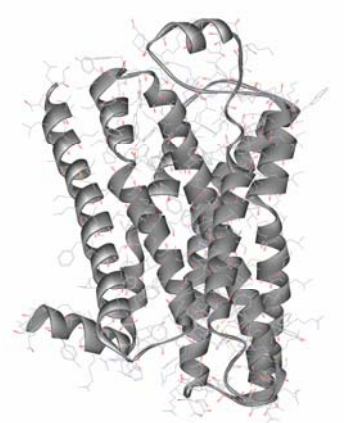

Fig. 2 – 3D-structure of alpha2A-AR obtained by homology modeling using the 2RH1 structure as a template.

From mutagenesis studies[30] the amino acid residues implicated in ligand binding have been determined: Asp3.32 (Asp113) Ser5.42 (Ser200) and Ser5.46 (Ser204). They are shaded in Table 1. In order to validate the model we used the docking software GLIDE[31] to dock norepinephrine, the endogen ligand of all adrenergic receptors. A grid that included all protein has been used for docking the norepinephrine. In 6 of 10 runs norepinephrine was docked in the ligand binding domain. One model is displayed in Fig. 3.

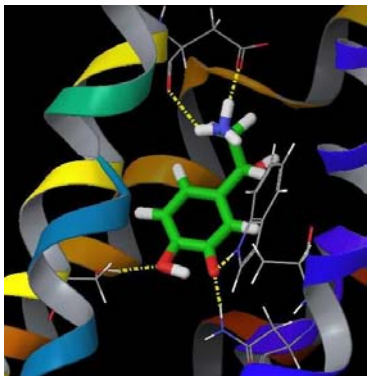

Fig. 3 – Norepinephrine docked in the 3D model of the alpha2A-AR.

In Figure 3 one can see interactions of two H atoms of the norepinephrine protonated N atom bound by H bonds to Asp3.32; also, the O atom of the 4-OH group of norepinephrine is implicated in an H bond with the H atom of the OH group of the Ser5.42 residue. Both geometry checking and endogen ligand docking validate the refined 3D model of the human alpha2A-AR obtained based on the X-ray structure of the human beta2-AR.

CONCLUSIONS

The three-dimensional model of the human alpha2A-AR was obtained as an attempt to have a better view of the ligand behavior in the binding site. The homology modeling of the human alpha2A-AR was possible because the human alpha2A-AR has similar structural pattern (seven transmembrane helices) to beta2-AR and highly conserved residues in the rhodopsin family. The obtained 3D model can be a useful tool for the design of new receptor mutants and hopefully, it might serve to design compounds with better selectivity for alpha2-AR subtype.

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