ABSTRACT
The transition of a G protein-coupled receptor (GPCR) from its inactive to the active state that couples to G proteins, involves rearrangements in transmembrane segments 3 and 6 (TMS3 & TMS6). We carried out molecular dynamics simulations of a validated model of the $5HT_{2C}$ subtype to study conformational changes that may constitute a molecular mechanism by which agonist binding triggers receptor activation. Our results focus on changes related to ligand binding involving the cluster of aromatic residues in TMS6 (Phe6.44, Trp6.48, Phe6.52) which straddles the absolutely conserved proline P6.50. Our findings point to the interaction between the aromatic moieties of Phe6.52 and 5HT as a trigger for the conformational change in the highly conserved Trp6.48. A change in the orientation of this residue from "perpendicular" to "parallel" to the membrane plane has been shown from spectroscopy to occur in the transition from inactive to activated rhodopsin. This rearrangement affects the bending angle of TMS6 that is caused by the proline-kink from P6.50. This coincides with conformational changes in TMS6 described experimentally to be associated with activation when TMS6 "moves away" from TMS3. Thus, a spatially ordered sequence of structural rearrangements stabilized by the interaction of the ligand with residues in the "binding pocket" suggests a molecular activation mechanism.

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A SPATIALLY ORDERED SEQUENCE OF INTRAMOLECULAR REARRANGEMENTS OBSERVED FROM SIMULATIONS OF AGONIST-RELATED ACTIVATION OF $5HT_{2C}$ RECEPTORS

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INTRODUCTION

The 2C subtype of serotonin receptors ($5HT_{2C}$) is a member of the G protein coupled receptor (GPCR) superfamily of seven transmembrane helix proteins. The cascade of events in which a GPCR changes from its inactive (R) to its active state (R*) has not yet been elucidated. The efficacy of agonists, partial agonists, antagonists and inverse agonists in eliciting responses from binding to GPCRs relates to their ability to modify the equilibrium between R and R* stabilizing one or the other to a different extent. Binding of the endogenous ligand 5Ht to its 5HT2C receptor has been shown previously to involve residues D3.32 [1], S3.36 [2] and F6.52 [3] (Figures 1 and 2).

The propagation of the ligand binding signal through the receptor structure, involves a "sensor" of its interaction at the binding site. Residue F6.52 is a candidate for this function by virtue of its position in a cluster of aromatic residues (F6.52, W6.48 and F6.44) in helix 6 that has been shown to be accessible to MTS reagents [4]. The "aromatic cluster" straddles the absolutely conserved proline P6.50, a change in the orientation of W6.48 from "perpendicular" to "parallel" to the membrane plane has been shown from spectroscopy to occur in the transition from inactive to activated rhodopsin [5]. Other conformational changes in TMS6 [6] [7], as well as in TM3 [8] [9] have been described experimentally to be associated with activation We find that a spatially ordered sequence of structural rearrangements stabilized by the interaction of the ligand with residues in the "binding pocket" and involving the "aromatic cluster" suggests a molecular activation mechanism that takes the receptor form the R state to R*.

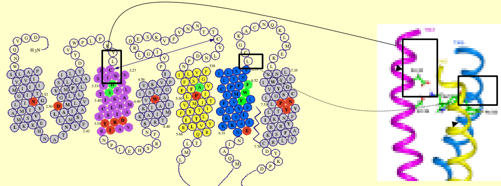


FIGURE 1: 5HT2C helical net. Residues highlighted in green are in direct contact with 5HT, and in the aromatic cluster in TM6. Highlighted in red are the main residues involved in the activation mechanism, or known to be essential in the bundling of the TM helices.

FIGURE 2: $5HT_{2C}$ residues in direct contact with 5HT.

METHODS

The construction of the model used the helix axes of the template described by Baldwin[10] [11] following the rhodopsin electronmicroscopy map of 7.5Å resolution. The transmembrane bundle incorporates the predictions from biophysical criteria presented in detail [12] that were used to identify the span of all the TM segments and their inward/outward orientation, as well as the experimental constrains that include information about ligand interaction at positions D3.32 [1], S3.36 [2], F6.52 [3], the role and position of the arginine cage at the cytoplasmic end of TM3 [9], and the specific structural information from experimental exploration of the water accessible faces of TM helices using substituted cystein accessibility method of TMHs 3, 4, 5, 6 and 7 [4] [13] [14] [15] [16][17].

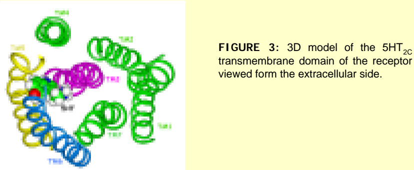
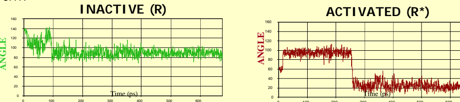


FIGURE 3: 3D model of the $5HT_{2C}$ transmembrane domain of the receptor viewed form the extracellular side.

Minimization and molecular dynamics simulations (MD) of the receptor and the ligand/receptor complex were performed using CHARMM 24 [Brooks, 1983 #496] with Charmm 22 parameters. The leap-frog algorithm was used for the dynamics with SHAKE constrains, a distance dependent dielectric constant, a time step of 0.001 and an update frequency of 5 for the non-bonded list. The MD simulation consisted of 658ps of equilibration and 200ps of production run at 300?K for the unoccupied receptor, and 338ps of equilibration and 325ps of productive run for the occupied receptor.

FINDINGS:

1. During MD simulations the angle between W6.48 and the plane of the membrane is calculated relative to the plane defined by the geometrical centers of helices 3, 4 and 6. Graph 1 represents this angle in the receptor alone, and Graph 2 in the receptor complexed with 5HT.

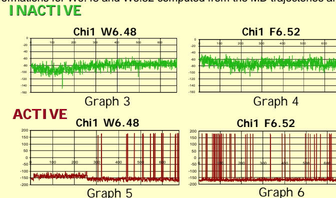


Graph 1

Graph 2

Graph 1 and 2: In the absence of 5HT (inactive) the angle between W6.48 and the plane of the membrane stabilizes around 90?, whereas in the presence of 5HT the angle oscillates at the beginning of the simulation around 90, to changes abruptly at 250ps to 35?, and stabilizing around 20?. The presence of the agonist in the binding pocket stabilizes a conformation of W6.48 that is in agreement with the transition described experimentally. W6.48 is not in direct contact with 5HT, what makes necessary the presence of a sensor that "detects" the presence and orientation of the ligand in the binding site. F6.52 in direct contact with the ligand and just four residues before in helix 6, is likely to be such a sensor.

2. The side chain conformations for W6.48 and W6.52 computed from the MD trajectories are shown in graphs 3-6.



Graph 3

Graph 4

Graph 5

Graph 6

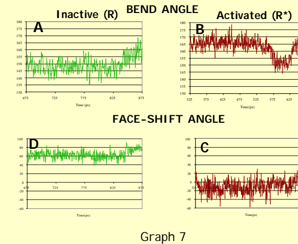
A **B**

The Chi1 angles for W6.48 and F6.52 the trajectory oscillate around a ***g+*** orientation in the unoccupied receptor (graph 3 and 4 respectively), while in the presence of 5HT both angles oscillate around ±180° (***trans***). These results indicate that the presence of the ligand stabilizes the Chi1 ***trans*** conformation for both W6.48 and F6.52, positioning W6.48 in the parallel orientation with respect of the plane of the membrane that is associated with the R* form of the receptor [5]. In the absence of 5HT a ***g+*** conformation is preferred, resulting in the perpendicular orientation of W6.48 with respect to the membrane associated with the inactive form R of the receptor (Figure 4 A and B).

FIGURE 4A : F6.52 and W6.48 Chi1 angles are stabilized in the ***trans*** conformation when 5HT is present, resulting in an orientation of W6.48 ***parallel*** to the membrane.

FIGURE 4B : F6.52 and W6.48 Chi1 angles are stabilized in the ***g+*** conformation in the absence of 5HT, resulting in an orientation of W6.48 ***perpendicular*** to the membrane.

3. Residues F6.52 and W6.48 straddle the conserved proline P6.50. We find that the conformation of those two residues is related to the kink induced in the helix by P6.50. The proline related distortion of the helix is characterized by two angles: the **bend** and the **face-shift**. The **bend** angle is between the axes of the helix preceding and following proline. In an ideal alpha helix residues i, i-3 and i-4 are on the same face of the helix. The proline induced distortion of the helix may also alter this regular pattern. The **face-shift** angle measures this distortion. Values of the face-shift angle close to 0? indicate a small distortion [17]. Graph 7 show the computed bend and face-shift angles computed for the receptor (inactive) and the receptor complexed with 5HT (active).



Graph 7

The **bend** angle and the **face-shift** induced by P6.50 are smaller in the presence of 5HT than in its absence. This indicates that a ***trans*** conformation of F6.52 and W6.48 favors a less kinked helix than a ***g+*** one. Thus the presence of the ligand in the binding site stabilizes a conformation of the receptor in which TM6 is minimally kinked, whereas in its absence a highly kinked TM6 is preferred.

4. Finally the change in the proline kink induced by P6.50 induces an increase in the distance between the cytoplasmic ends of TM3 and TM6, such as reported to occur upon activation from EPR measurements on rhodopsin [8] [9] (figure 5).

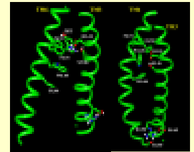


Figure 5

CONCLUSIONS:

1. The presence of 5HT in the binding site stabilizes an orientation of W6.48 parallel to the membrane that is associated with the R* form of the receptor [5], while in its absence W6.48 prefers the perpendicular orientation associated with the R form [5].
2. The effect of 5HT on the orientation of W6.48 is transmitted through the "aromatic cluster". The "sensor" of 5HT binding is F6.52 which is preferentially in a ***g+*** rotamer of Chi1 in the unoccupied receptor, but adopts Chi1=***trans*** as a result of interaction with 5HT. R The reorientation of F6.52 and W6.48 are fully coordinated within the "aromatic cluster".
3. The coordinated conformational changes of F6.52 and W6.48 affect the distortion induced by P6.50 in the helix: the conformation adopted by the "aromatic cluster" in the presence of 5HT (in the R* form) is associated with a reduction of the kink observed in the helix for the R form.
4. The calculation of **bend** and **face-shift** associated with the Pro-kink have proved to be a highly sensitive measure of the dynamic behavior of the Pro-induced distortion during MD trajectories.
5. The experimentally observed increase in distance between TM3 and TM6 cytoplasmic ends can be related to the hinge motion around P6.50. This motion is induced by the conformational change of the two straddling residues F6.52 and W6.48 of the "aromatic cluster", from ***g+*** (R form) to ***trans*** (R* form). This conformational change is triggered by the interaction of the ligand (5HT) with the sensor (F6.52).

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