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Fully flexible docking models of the complex between α7 nicotinic receptor and a potent heptapeptide inhibitor of the β-amyloid peptide binding

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Abstract—The heptapeptide IQTTWSR (IQ), recently reported as inhibitor of the β-amyloid (Aβ) binding to nicotinic acetylcholine receptors (nAChrs), was docked to the homology model of the α7 nicotinic acetylcholine receptor. The most representative models were further subjected to molecular dynamics simulations. The data obtained here suggest that Aβ needs highly specific structural motifs to bind to the α7nAChR. These structural motifs are located principally in the upper and lower surroundings of loop C, including loop F and sheets β1, β2, β6, β9, and β10 of the receptor. Overall, these results suggest that IQ can be mimicked by more bioavailable, stable compounds that would be helpful for the understanding of the Aβ binding site and its dynamics, and for the design of novel agents to be used as an effective alternative against Alzheimer's disease.

Alzheimer's disease (AD) is a progressive neurological disorder that is characterized by memory loss. Currently, AD affects nearly 2% of the population in industrialized countries. Its pathogenesis has been attributed to the malfunction of different but related pathways of brain cells.[1] Among these, it has been shown that the accumulation of the β-amyloid peptide (Aβ) is a primary event in the pathogenesis of the AD.[2] This 42-amino acid, 4 kD peptide is derived from the proteolytic cleavage of the Aβ precursor protein.[3] Aβ has been shown to be neurotoxic due to its interaction with different receptors, including its own aggregation in neurons, astrocytes, and microglia.[4–7] Recently, the Aβ interaction with nicotinic acetylcholine receptors (nAChRs), and particularly with the homomeric α7 nicotinic acetylcholine receptor (α7nAChR), has been shown to contribute to the impairment of cognitive abilities, which is characteristic of the AD.

α7nAChR is widely expressed in the central and peripheral nervous systems and plays an important role in many calcium-dependent processes by regulating the flux of this ion.[8–10] The entry of calcium during temporally associated dendritic action potentials and synaptic potentials is a key factor in the development of the AD. It has been demonstrated that the Aβ co-immunoprecipitates with high affinity with the α7nAChR in samples from post-mortem AD hippocampus and the antagonists of this receptor compete with Aβ.[11,12] Furthermore, the effect of the Aβ on stratial cholinergic neurons, which express very low levels of α7nAChR, was found as not significant.[13] Aβ has also been shown to reduce α7nAChR-mediated current in cultured cortical neurons, leading to alterations in the calcium influx.[14] Additionally, Aβ and its fragment $A\beta_{12-28}$ reduced carbachol-induced currents in stratum radiatum interneurons in hippocampal slices via a decrease in the probability of α7nAChR-gated channel opening.[15] Thus, the α7nAChR has received significant attention as a promising target to treat the AD.

Docking models of the Aβ and Aβ fragments on the ligand-binding domain of the α7nAChR have provided a good starting point for the localization of the Aβ and its influence on the dynamics of the α7nAChR.[16,17]

Keywords: Alzheimer's disease; α7 Nicotinic acetylcholine receptor; β-Amyloid peptide; Docking; Molecular dynamics simulations; Drug design.
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Additionally, Henchman et al.[18,19] as well as Law et al.[20] have performed molecular dynamics simulations of the ligated and unligated human α7nACh; these studies have provided the basis for understanding the dynamics and gating mechanism of this receptor at atomic level, which would be very useful to understand how Aβ could affect the overall dynamics of the α7nAChR. Unfortunately, the Aβ–α7nAChR complexes previously reported were obtained by means of fully rigid docking (i.e., no torsional degrees of freedom were assigned either to the receptor or to the peptide), due to the high complexity of the systems.[16,17]

Recently, Ferreira and co-workers found that the heptapeptide IQTTWSR (denoted as IQ), which is homologous to the region Q54-D60 (β2 sheet) of the acetylcholine binding protein (AChBP), is able to inhibit the binding of $A\beta_{1-40}$ to the α7nAChR by attaching itself to the receptor.[21] Furthermore, kinetic whole-cell current-recording measurements showed that Aβ inhibited nAChR function in a dose-dependent manner in neuronal differentiated PC12 cells and IQ completely blocked the formation of the Aβ–α7nAChR at nanomolar concentrations.

Building on the study performed by Ferreira and co-workers and the previously reported models of the complexes between Aβ and Aβ fragments and the α7nAChR,[16,17] in this contribution two dynamic models between the peptide IQ and the α7nAChR are reported. These models were obtained through robust, fully flexible docking simulations of the peptide over the rigid ligand-binding domain of the α7nAChR using the well-known program AutoDock.[22] Furthermore, the two models obtained here were optimized through 5-ns molecular dynamics simulations. These models showed two binding sites are related to the Aβ binding to the α7nAChR. Both binding sites reported here are located very close to the acetylcholine binding site and the mobile loop C; the latter has been shown to play an important role in the dynamics of the binding site and therefore in the global dynamics of the receptor.[18,19] These simulations would provide valuable insights for a better understanding of the regions of the α7nAChR implicated in the formation of the Aβ–α7nAChR complexes. Thus, these findings would be particularly useful to design novel agents that block the Aβ binding to the receptor and therefore improve the memory impairments that are characteristic of the AD.

Flexible docking of the peptide IQ on the human α7nAChR ligand-binding domain was performed with the aid of the program AutoDock. Hetenyi and van der Spoel demonstrated that AutoDock is very efficient in locating the binding modes of peptides on proteins without prior knowledge of the binding site ('blind' docking approach).[23] However, in this study a 'semi-blind' docking approach was employed, and only a large, but specific, region of the ligand-binding domain of the receptor was used. This region, comprised only by two symmetric subunits of the receptor, was chosen taking into consideration the results obtained in two previous docking studies[16,17] and the experimental data obtained by Ferreira and co-workers.[21,24]

The first orientation obtained in the present study (Fig. 1, top) showed that IQ bound mostly to the loop F of the receptor, but it also interacted with the β9 sheet and with the lower region of the β10 sheet. (Throughout the manuscript, this binding site will be labeled as IQ-1.) It was also observed that IQ showed minor interactions with loop C. The most important contacts of individual residues which belong to IQ along its binding site are listed as follows: I1 interacted with residues Y167, I168, P169, and D174; only two residues, R204 and T207, made contact with residues Q2 from IQ, while residue T3 had no contacts with the receptor. Residues T4 and S6 interacted only with one amino acid each one (P169 and S165, respectively), while W5 had several contacts along the IQ-α7nAChR interface, principally with residues I164, Y167, L175, and V176 from the (−)-α7 subunit. Finally, R7 had contacts with E161 from the (−)-α7 subunit and with E188 and C189 from the (+)-α7 subunit.

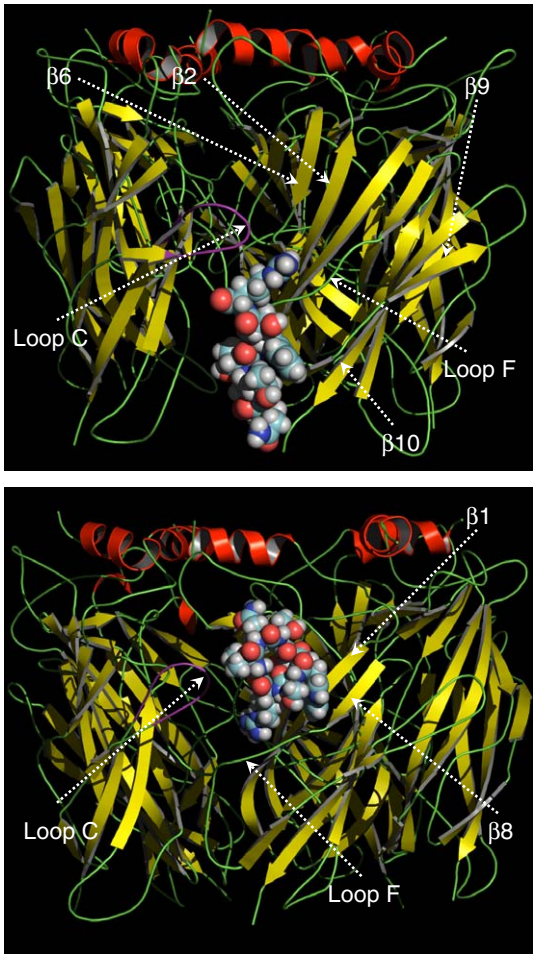

Figure 1. Two most representative orientations of the peptide IQ on the α7nAChR. Top: location of IQ at site IQ-1; bottom: location of IQ at site IQ-2. IQ is shown as van der Waals spheres, while the receptor is shown as cartoons.

In contrast, the second orientation (Fig. 1, bottom) found by using combined docking/molecular dynamics simulations placed IQ in the zone comprising the upper regions of loop F, β1, β2, and β6 sheets; some contacts were also observed with the sheet β8 and with loop C (This binding site will be referred to as IQ-2.) In this case, the contacts between IQ and the α7nAChR in this binding site were not as extensive as those observed in the first one. When IQ docked to the site IQ-2, only residues Q2, T3, T4, W5, and R7 from the peptide were shown to interact with different residues from the receptor, while residues I1 and S6 were exposed to the solvent. The most important contacts per residue are described as follows. T3 (IQ) interacted with residues T60 and S112 from the (−)-α7 subunit, while residue T4 made contact with T60, Y31, H114, and Q160, all from the same subunit. Residues Y31, Q158, M159, and Q160 ((−)-α7 subunit) were responsible for binding residue W5 from IQ, while D163, Q190, and E191 from the (−)-α7 subunit and R188 from the (+)-α7 subunit interacted with residue R7 from IQ. Finally, residue Q2 only interacted with residue S112 from the (−)-α7 subunit.

It was observed that at least three types of interactions along both binding sites play an important role in binding and stabilizing the complexes between IQ and the receptor. However, their level of contribution is different for the two sides. To better illustrate the contribution of different interactions to bind the peptide to the receptor, the frequency of van der Waals/hydrophobic as well as electrostatic interactions (hydrogen bonds and salt bridges) were analyzed at the interface of each complex. In this study, it was found that van der Waals/hydrophobic contributions that lead to the binding of IQ to the receptor were similar for the two binding sites. However, it was not the case for hydrogen bonding and electrostatic contributions. Several differences between the number of hydrogen bonds and salt bridges were observed when IQ-1 and IQ-2 were compared. Tables 1 and 2 show that the amount of hydrogen bonds observed for the IQ-α7nAChR at 5 ns is significantly different between the two binding sites (see Supporting materials). For example, residues Q2, W5, and R7 from IQ, form a total of 13 hydrogen bonds at the site IQ-1, while only two residues from IQ, T4, and R7, display six hydrogen bonds at site IQ-2.

Several salt bridges are observed at the interface of the complexes. Residue Q2, that belongs to IQ, displays two salt bridges with D38 and R185 from the (+)-α7 subunit, while R7 interacts with E188 ((+)-α7 subunit) and E161 ((−)-α7 subunit). Similarly, R7 from IQ forms salt bridges with E188 ((+)-α7 subunit) and D163 ((−)-α7 subunit).

Given the nature of hydrogen bonding and salt bridges and their contribution to the binding of IQ to either of the sites IQ-1 and IQ-2, it can be inferred that these types of interactions strongly affect not only the specific binding of IQ to the receptor, but also its stabilization and the dynamics of the α7nAChR. However, at this point further research will be needed to determine their importance, because the model presented here is limited only to locate the binding sites for IQ and its binding modes on those sites.

The dynamic models of the IQ-α7nAChR complex presented here reveal that there are two potential binding sites that can be targeted to avoid the Aβ binding to the receptor. Interestingly, both binding sites are very close to each other and occur in the interface formed by the (+)-α7 and (−)-α7 subunits. Both were found to interact with Aβ, as revealed in a previous study.[16]

The results or findings of the present study are also supported by experimental data obtained by Ferreira and co-workers,[21] and by the recent molecular dynamics simulations performed by Henchman et al.[18,19] Ferreira and co-workers demonstrated that IQ, which closely mimics the upper region of the β2 sheet of the α7nAChR, was able to specifically block the inhibition of nAChRs by $A\beta_{1-40}$ at nanomolar concentrations of the peptide and that it prevented $A\beta_{1-40}$-induced cell death in PC12 cells. These authors suggest that the peptide can either work as a competitive ligand at the $A\beta_{1-40}$ binding site or as an allosteric modulator. Interestingly, the present study finds that both effects are possible, considering the structural dynamics displayed by the receptor when IQ is bound to either IQ-1 or IQ-2 site.

In two molecular dynamics studies recently performed by Henchman et al., some of the local and global dynamics of the receptor were revealed.[18] It was observed that, when the free ligand-binding domain of the receptor was subjected to molecular dynamics simulations, only few regions of the receptor were flexible. These regions included the Cys loop, and loops B and C; on the other hand, regions such as loops D–F, and the sheets β1–β10 remained fairly rigid throughout the simulation. In this study, it was observed that site IQ-1 was mainly formed by loop F and sheets β9 and β10, while loop F, and β1, β2, and β6 sheets form the site IQ-2; however, some residues from sheet β8 and loop C play also an important role in binding IQ to the latter.

Considering these findings, it can be hypothesized that the most rigid regions of the receptor seem to be a good target for a small peptide such as IQ. It appears that at very low concentrations the peptide does not affect the normal activity of the receptor. This is revealed by the rapid kinetic whole-cell current-recording measurements, which showed that Aβ inhibited nAChR function in a dose-dependent manner in neuronal differentiated PC12 cells and that nanomolar concentrations of IQ completely blocked the inhibition by Aβ. Taking into account that the two potential regions where IQ binds remain dynamically stable, it might be possible that this peptide does not interfere with the natural dynamics of the receptor, and it will be able to prevent Aβ from binding to the receptor.

In a previous study where rigid docking of the Aβ was performed on the α7nAChR, it was observed that the peptide interacted with both binding sites here

described.[16] In addition, four Aβ fragments, $A\beta_{1-11}$, $A\beta_{10-20}$, $A\beta_{12-28}$ and $A\beta_{22-35}$, were also docked to the α7nAChR. Strikingly, all peptides bound to the same binding site; this binding site corresponds to the IQ-2 in the present study. Particularly, when $A\beta_{10-20}$ and $A\beta_{12-28}$ bound to the receptor, one of the peptide termini interacted with some regions of the loops C and F.[17]

Following both theoretical and experimental data, it can be hypothesized that IQ is not recognized by a single site of the α7nAChR. Instead, at least two binding sites are involved in the recognition of the peptide. Thus, a very low concentration of the peptide would be enough to protect the receptor from the Aβ.

Considering that both sites IQ-1 and IQ-2 are dynamically quite stable (e.g., their rigid-body fluctuations are small), it is possible that their structure would be similar in both desensitized and activatable states of the receptor. It was also observed that the location of IQ on both of its sites on the receptor did not significantly affect the motions of loop C, which are important for the binding of the acetylcholine. Finally, the binding sites found in this study correlate well with that observed for the Aβ and its fragments.[16,17]

Therefore, the mechanism in which this peptide might act may be hypothesized as follows: in theory, IQ could bind either to one or both sites described here. The binding process could be given over desensitized and activatable states of the receptor, and it is possible that its natural motions (e.g., the opening–closing motions of loop C) will not be affected by IQ. After binding, IQ would 'protect' some of the regions where Aβ binds. Finally, Aβ has less possibilities of binding to the receptor, since it would require specific arrangement and composition of its binding face at the α7nAChR to dock to the receptor. This means that Aβ will not be able to 'recognize' its binding site, and consequently it will not be able to bind to the receptor.

This hypothesis raises an interesting point about the selectivity of the receptor to bind Aβ. When any of the two binding sites on the α7nAChR that could be targeted is occupied by this heptapeptide, there is a higher probability that Aβ is not going to bind to the receptor. Thus, Aβ will require a complete, large, and very specific region of the α7nAChR to bind and present its activity on the receptor. This observation is supported by data obtained by Wang et al.[12] In their study, kinetics for the binding of Aβ to α7nAChR were determined using the subtype-selective nicotinic receptor ligands [^{3}H]methyllycaconitine and [^{3}H]cytisine. It was found that Aβ binds (bound) with a very high affinity to the receptor (K_i values of 4.1 and 5.0 pM for rat and guinea pig receptors, respectively). These findings, together with the fact that IQ is a weaker agent (~1000 times less than Aβ),[21] lead us to suggest that a representative change in the Aβ binding site is enough to inhibit the binding of Aβ to the α7nAChR. This also supports the hypothesis that IQ competes with Aβ for the same binding site(s).

It was also suggested by Ferreira and co-workers that IQ might bind to an allosteric site in the receptor, thereby inducing a conformational change of the nAChR, which prevents Aβ binding. It is possible that some allosteric sites could be found in the 'external' face of the receptor, where IQ binds. For example, the location of multiple and distant allosteric sites has been recently reported for the M_1 muscarinic acetylcholine receptor.[25,26] At this stage, the hypothesis that IQ's main function is to block the Aβ is in agreement with the experimental data as well as with the structural data presented here.

An important issue that will be explored in future studies is the overall dynamic behavior of the receptor observed in this study. The initial structure of the α7nAChR with IQ docked in both sites IQ-1 and IQ-2 was the same. Interestingly, after 5 ns of simulations, the receptor showed a significantly different dynamical behavior. As observed in Figure 2 (top), the structure of α7nAChR when the site IQ-1 is occupied by the heptapeptide resembles the structure observed for the complex between acetylcholine and the receptor. In contrast, when IQ binds to the site IQ-2 (Fig. 2, bottom), the dynamics displayed by the receptor are very similar to what is observed for the α7nAChR in its apo form and when it is bound to an antagonist (d-tubocurarine).

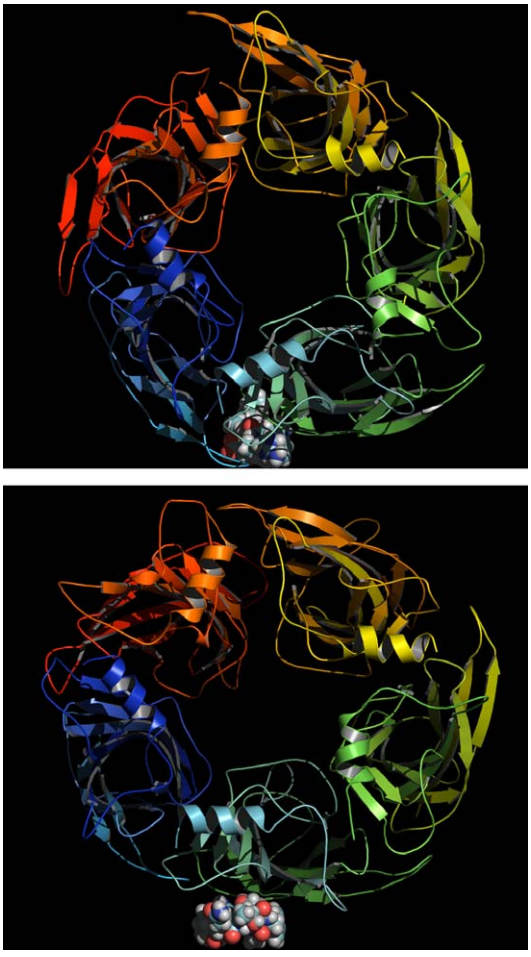

Figure 2. Final structures after 5 ns for the complexes IQ-α7nAChR at sites IQ-1 (top) and IQ-2 (bottom). The receptor is shown as cartoons and IQ as van der Waals spheres.

Henchman et al. noticed that, in the presence of acetylcholine, the receptor remained more open and displayed more symmetric arrangement of the five subunits, whereas a more closed and asymmetric arrangement results for the apo and d-tubocurarine cases.[19] Here, the behavior observed for the receptor bound to IQ can be artificial due to the fact that only one site of the receptor is occupied by IQ and that it is studied in its apo form. Another important factor that may be crucial in determining the complete effect of this peptide over the receptor is the length of the simulations and that the docking/molecular dynamics simulations were performed only using the ligand-binding domain of the receptor (i.e., the transmembrane domain was not included in our model). Thus, more detailed simulations need to be performed, including acetylcholine and the saturation of all possible binding sites of the receptor where IQ can bind.

In summary, two binding sites on the α7nAChR, IQ-1 and IQ-2, were found to bind the peptide IQ, a novel agent that has been experimentally shown to inhibit the binding of $A\beta_{1-40}$ to this receptor. The location of both binding sites is in agreement with recent molecular dynamics simulations performed on the receptor and rigid docking simulations of Aβ, where more stable regions of α7nAChR will serve as well-defined binding sites.

Additionally, the results obtained in this study suggest that Aβ needs highly specific structural motifs in order to bind to the α7nAChR. These structural motifs are located principally in the upper and lower surroundings of loop C, where Aβ was initially found to bind.[16]

Considering these results, further experiments can be suggested. For example, mutagenesis analysis of some of the residues that belong to loop F and the β-sheets surrounding the regions mentioned above could be performed. Subsequently, the kinetics of binding between the receptor and Aβ could be recorded. In addition, simulations of the α7nAChR in complex with acetylcholine and IQ would provide a better view of the effect of IQ on the overall motions of the α7nAChR. These simulations would facilitate the design of novel compounds that are able to mimic IQ.

While more experimental and theoretical work needs to be performed, this study would be helpful for the design of agents that mimic IQ. Such agents could be used not only as a protective agent of the receptor, but also to potentially attack other targets related to the disease, such as the inhibition of the formation of Aβ plaques (multi-target approach).[27]

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2006.03.093.

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