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Molecular dynamics at the receptor level of immunodominant myelin oligodendrocyte glycoprotein 35–55 epitope implicated in multiple sclerosis

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ABSTRACT

Multiple Sclerosis (MS) is a common autoimmune disease whereby myelin is destroyed by the immune system. The disease is triggered by the stimulation of encephalitogenic T-cells *via* the formation of a trimolecular complex between the Human Leukocyte Antigen (HLA), an immunodominant epitope of myelin proteins and T-cell Receptor (TCR). Myelin Oligodendrocyte Glycoprotein (MOG) is located on the external surface of myelin and has been implicated in MS induction. The immunodominant 35–55 epitope of MOG is widely used for *in vivo* biological evaluation and immunological studies that are related with chronic Experimental Autoimmune Encephalomyelitis (EAE, animal model of MS), inflammatory diseases and MS. In this report, Molecular Dynamics (MD) simulations were used to explore the interactions of MOG_{35-55} at the receptor level. A detailed mapping of the developed interactions during the creation of the trimolecular complex is reported. This is the first attempt to gain an understanding of the molecular recognition of the MOG_{35-55} epitope by the HLA and TCR receptors. During the formation of the trimolecular complex, the residues Arg^{41} and Arg^{46} of MOG_{35-55} have been confirmed to serve as TCR anchors while Tyr^{40} interacts with HLA. The present structural findings indicate that the Arg at positions 41 and 46 is a key residue for the stimulation of the encephalitogenic T-cells.

1. Introduction

Multiple Sclerosis (MS) is the most common autoimmune disease of the Central Nervous System (CNS) in which a coordinated attack of the immune system against myelin takes place [1,2]. Myelin Basic Protein (MBP), Proteolipid Protein (PLP), Myelin Oligodendrocyte Glycoprotein (MOG) and Myelin-Associated Glycoprotein (MAG) are the main myelin proteins in the CNS and they have been recognized as putative auto-antigens for MS [3]. MS onset is triggered by the activation of the encephalitogenic T-cells through the formation of a trimolecular complex between the T-cell receptor (TCR), the peptide (antigen) –with identical residue sequence to a fragment of a protein of the myelin sheath (molecular mimicry theory) [4,5] and the Major Histocompatibility Complex (MHC) or Human Leukocyte Antigen (HLA). The ability of the peptide-HLA complex to activate T-cells is correlated with the strength of its binding affinity with TCR [6–8]. Conversely, stimulation or not of T-cells that are responsible for MS follows [9–13]. The HLA class II receptors are dimers comprised of two different polypeptide chains (α and β) [14,15]. In this class of macromolecules the polypeptide chains are joined together, creating a single receptor for the antigens to bind to. Subsequently, the newly formed complex is recognized by the receptors on the surface of T-cells. The formation of the trimolecular complex leads to the activation of the T-cells through a cascade of biochemical changes and the induction of the immune response to the antigen. The TCR is also composed of two different polypeptide chains. The two chains comprising the TCR (α and β), include variable domains called Complementarity Determining Regions (CDRs). These domains are implicated in the recognition process between the TCR and the HLA-antigen complex [16]. The diversity of the CDRs plays a crucial role in the recognition of the different antigens as they are presented by the HLA receptors. The CDRs comprising the TCR chains are very effective at screening the various antigens presented to the T-cells [9,17]. It has been estimated by *in vivo* experiments that the TCR unique structures in humans are over $>2.5 \times 10^7$ [17,18]. Despite

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the diverse numbers and the rigorous selection process of T-cells in the thymus, [19,20] there are cases where the selection process fails to single out T-cells reacting to self-antigens [21]. Thus, the failure in the thymic selection leads to self-reactive T-cells and induction of autoimmune disorders such as MS [22,23].

The myelin sheath consists of multiple proteins; MBP and PLP are two of the major component of the myelin sheath, while MOG and Myelin-associated Glycoprotein (MAG) are less abundant [24,25]. The total myelin proteins consist of only 0.05%–0.1% of MOG; [24,25] however MOG or MOG epitopes activate T-cell immune responses in Experimental Autoimmune Encephalomyelitis (EAE, animal model of MS) and have also been associated with MS induction [26,27]. There have been extensive studies of different fragments of MOG as autoimmune triggers [28–32]. The MOG epitopes 1–22, 61–80, 92–106 have been highlighted as target antigens and the 35–55 epitope has been found to induce highly specific antibodies reacting similar to the entire MOG protein [33,34]. Moreover, T-cell responses to MOG epitopes have been measured through biological assays in MS patients [29,30].

The MOG_{35-55} epitope (M^{35}EVGWYR**S**^{42}PFSRVVHLYRNGK^{55}), based on the mouse/rat MOG peptide sequence, is shown as strongly immunogenic in mice leading to the development of chronic EAE [27,35]. Our group has previously rationally designed and synthesized linear and cyclic peptide analogues of human MOG_{35-55} immunodominant epitope ($hMOG_{35-55}$) with crucial TCR substitutions [26]. These altered peptides have proven to inhibit the clinical manifestation of symptoms of chronic EAE in mice [26]. The substitutions of Arg at positions 41 and 46 by Ala, result in peptide analogues that reduce the severity of MOG-induced EAE clinical symptoms in C57BL/6 mice when co-administered with mouse/rat MOG_{35-55} peptide at the time of immunization [26]. The observed results justify the importance of Arg at positions 41, 46 for EAE induction [26].

To the best of our knowledge no conformational study of $hMOG_{35-55}$ epitope in complex with TCR and HLA has been reported. Hence, this is the first attempt to provide a deeper understanding of the structural requirements in the trimolecular complex with $hMOG_{35-55}$ epitope. A detailed analysis of interactions between $hMOG_{35-55}$ and the respective receptors (HLA and TCR) could provide valuable information for rational design of altered peptide ligands (APLs) and non-peptide mimetics with inhibitory activity. Herein, the structural properties of $hMOG_{35-55}$ in three different environments were investigated, through the use of molecular dynamics simulations: i) we looked at the adopted conformations by the peptide in aqueous solution, ii) we carried out MD simulation studies using $hMOG_{35-55}$ in combination with HLA DR2 (DRA, DRB1*1501) receptor and iii) the created trimolecular complex between $hMOG_{35-55}$, HLA DR2 and TCR was explored. The investigation of the interactions between $hMOG_{35-55}$ and the receptors in the trimolecular complex is expected to provide important information on the binding patterns of the hMOG epitope, which can assist researchers in the rational design of novel molecules, focusing on the inhibition of the stimulated encephalitogenic T-cells that are responsible for EAE and MS induction.

2. Methods

The high resolution crystal structure of HLA DR2 (DRA, DRB1*1501) in complex with the MBP_{83-96} antigen (E^{83}NPVVHFFKNIVTP^{96}) and TCR was used for the MD simulations (PDB code: 1ymm) [36]. The $hMOG_{35-55}$ epitope (M^{35}EVGWYR**P**^{42}PFSRVVHLYRNGK^{55}), based on the human sequence, used for the simulation studies was constructed using PyMOL [37]. During this process the amino acids (L configuration), comprising the peptide, were placed in sequential order with no initial secondary structure assignment (unfolded conformation). The different systems were subjected to all-atom unrestrained MD simulations in explicit solvent using AMBER14 [38].

2.1. Molecular dynamics (MD) simulation of $hMOG_{35-55}$

For the construction of the $hMOG_{35-55}$ peptide parameters, the AMBER force field ff14SB [39] has been used. The TIP3 P water model [40] was utilized for the solvation of the system and the total charge was neutralized by the addition of three Cl^- ions. Truncated octahedral periodic boundary conditions have been applied to the system with a cutoff distance of 10 Å. The next step involved the minimization, followed by the heating of the system, under constant volume, to 300 K for 100 ps using the Langevin dynamics temperature scaling [41]. This was followed by equilibration for another 100 ps under constant pressure. Both heating and pressure equilibration were performed using a 10 kcal mol^{-1} $Å^{-2}$ restraint on the solute. The equilibration step under constant pressure was prolonged for a further 200 ps, after removing all restraints. The MD production run was performed under constant pressure and temperature conditions (NPT ensemble) for 100 ns. The temperature was kept constant with the use of the Langevin thermostat (using a collision frequency of 2 ps^{-1}). All bonds involving hydrogen atoms were kept to their equilibrium distance with the SHAKE algorithm (allowing for a 2 fs time step to be used) [42]. The long range electrostatic interactions were calculated with the Particle Mesh Ewald (PME) method [43].

2.2. Molecular dynamics (MD) simulation of the $hMOG_{35-55}$ complexed with the HLA DR2 receptor

The positioning of the $hMOG_{35-55}$ epitope inside the HLA DR2 receptor was performed manually using PyMOL [37]. The minimized structure (Section 2.1) of the linear $hMOG_{35-55}$ epitope was superimposed with the crystal structure of the MBP_{83-96} epitope (PDB code: 1ymm).[36] The orientation of $hMOG_{35-55}$ resembles the positioning of the MBP_{83-96} epitope inside the binding pocket of HLA DR2 [36,44]. The residues Tyr^{40}, Pro^{43}, Ser^{45} and Val^{48} of hMOG were placed in the respective pockets (P1, P4, P6 and P9 for HLA DR2), as reported in the literature [36,44,45]. In the next step, the residues comprising the TCR were removed along with all crystallographic water molecules. The missing hydrogens were added using the tLeap module in AMBER14.

As with the MD simulation of $hMOG_{35-55}$ in water, the parameters for the receptor were constructed using the AMBER force field ff14SB [39]. The total charge of the system was neutralized with the addition of fourteen Na^+ ions. The TIP3 P water model [40] was used in the solvation of the system and truncated octahedral periodic boundary conditions were applied to the system with a cutoff distances of 10 Å. The system was minimized over 5000 steps, followed by the gentle heating to 300 K over 100 ps, using the Langevin dynamics temperature scaling (time step 2 fs). The system was further equilibrated under constant pressure for 100 ps. As mentioned in Section 2.1, both the heating and equilibration steps were performed using a 10 kcal mol^{-1} $Å^{-2}$ restraint on the solute. A 200 ps equilibration step, under constant pressure, was performed following the removal of the restraint on the solute. Finally, an MD production run for 40 ns was performed using the NPT ensemble.

2.3. Molecular dynamics (MD) simulation of TCR-$hMOG_{35-55}$-HLA DR2 complex

For the MD simulation of the $hMOG_{35-55}$ epitope in complex with both TCR and HLA the construction of the system followed the same steps described in Section 2.2. The minimized conformation of the linear $hMOG_{35-55}$ epitope (Section 2.1) was superimposed with