Exploring Citrullination Effects on HLA-DRB1 Structure and Dynamics: Implications for Rheumatoid Arthritis

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Introduction

Citrullination is a post-translational modification (PTM) process which contributes to Rheumatoid arthritis (RA) development by modifying proteins in joint tissue. Anti-citrullinated protein antibodies (ACPA) positive RA patients exhibit a more severe form of the disease and are associated with some inherited risk HLA-DRB1 alleles, which encodes MHC class II molecules composed by a conserved amino acid sequence (QKRAA/QRRAA) in the peptide-binding groove named shared epitope (SE)[1]. These alleles can present citrulline-modified and unmodified peptides, but the citrullinated one results in an effector immune response in RA. In addition, the citrullinated residue in the peptide interacts with residue 71 of the SE which represents the pocket P4 of the MHC[2].

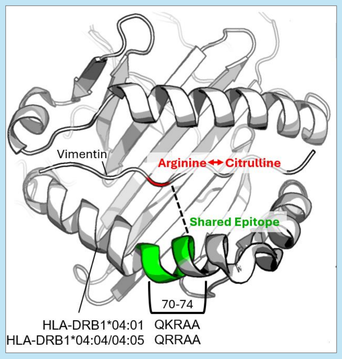

Figure 01: peptide-MHC (pMHC) 3D representation.

Aim

In this study, we intend to evaluate the intermolecular interactions between the HLA-DRB1 molecule (significant risk-RA-development allele) and peptide-ligands with or without PTM, using computational approaches.

Methods

3D structure (Crystal) of HLA-DRB1*04:01/*04:04/*04:05 complexed to a citrullinated peptide.
(**PDB ID:** 4MCY/4MD5/6BIR)

Protein Data Bank (PDB)[3]

Missing residues

CHARMM-GUI server [4]

Native pMHC modelling:
Cit<>Arg
Amino acid at the position 6 in the vimentin (Vim-p6)

Mutagenesis (PyMol v.2.4)

Molecular Dynamic (MD) simulation (4 replicates of 50 ns)

GROMACs v.2019

Analysis:
Root Mean Fluctuation (RMSF);
Autodock 4 (Binding Afinitty);
Hydrogen Bonds atom-pair calculation;
Coulombic energy estimation.

Results

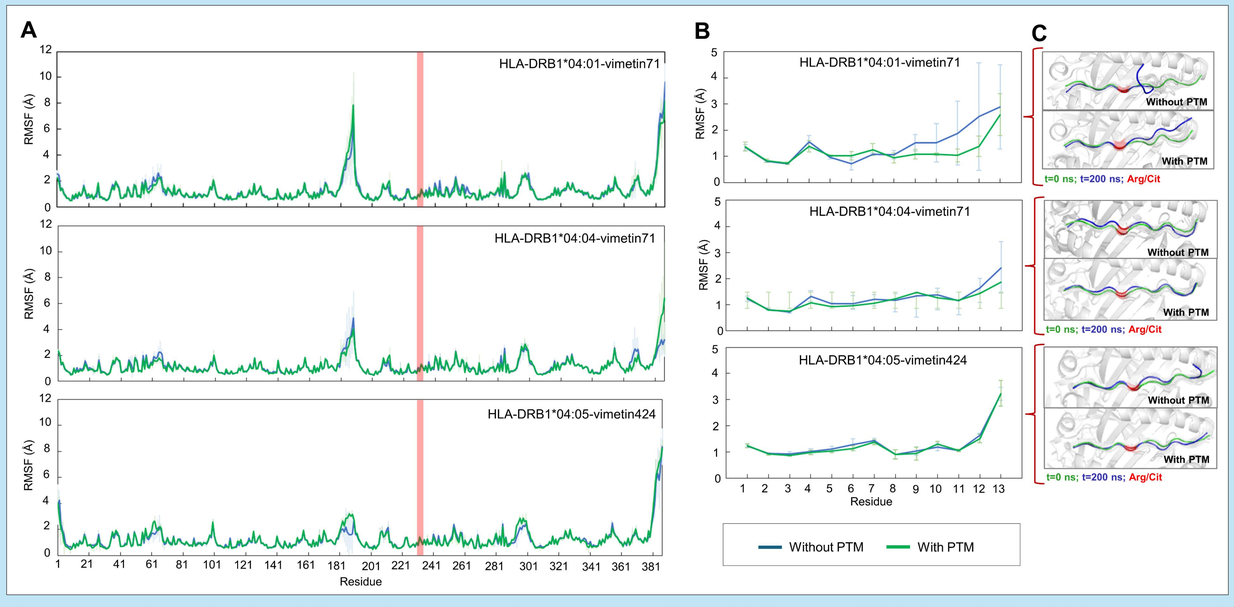

Figure 2: Conformational variations of pMHC complexes. RMSF values of (A) HLA and (B) vimentin (peptide) were plotted. Residues numbered from 1 to 89 belong to HLA-DRA1, 190 to 387 belongs to HLA-DRB1 and the red box indicates the shared epitope positions for each pMHC system in the (A) plot. The results consist of average and standard deviation calculations from the 4 replicate runs. **(C) Structural alignment between the initial (t=0ns) and final (t=200 ns) vimentin peptide MD 3D structures**. Replicates runs were concatenated in a unique long trajectory to perform this analysis. In red are highlighted the amino acid at the position 6 (Vim-p6) in the vimentin peptide.

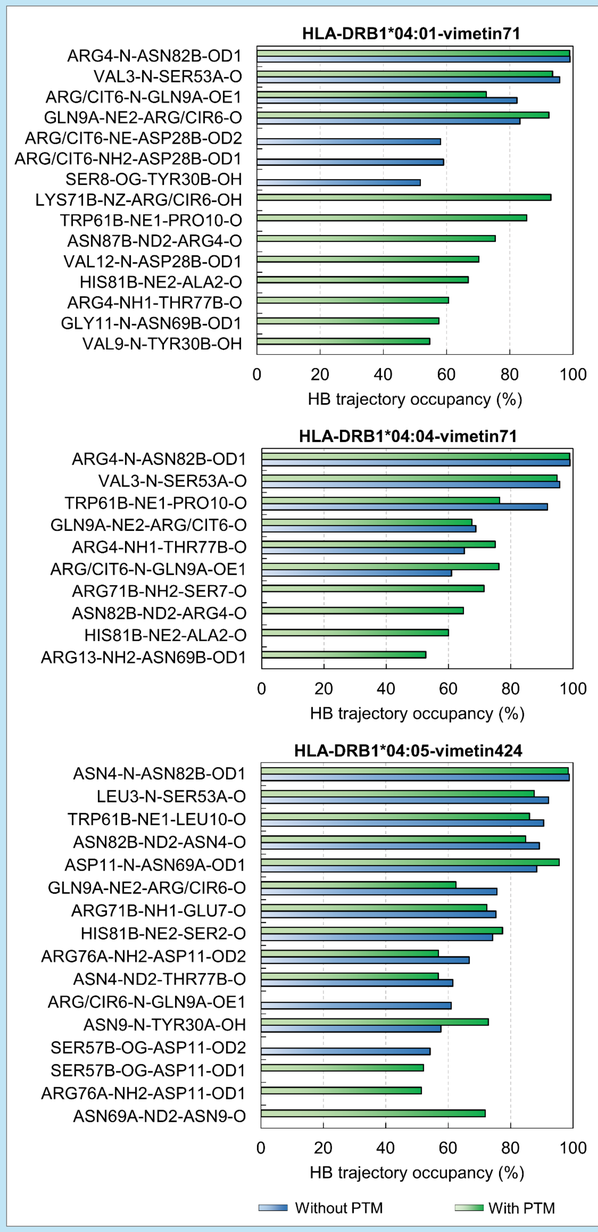

Figure 3: Hydrogen bond atom-pairs prevalence for each pMHC system during MD trajectory. Intermolecular hydrogen bonds with more than 50% prevalence are shown. The residues paired with the amino acid number, chain and atom are shown in the x axis refer to the amino acid-atoms donor and acceptor.

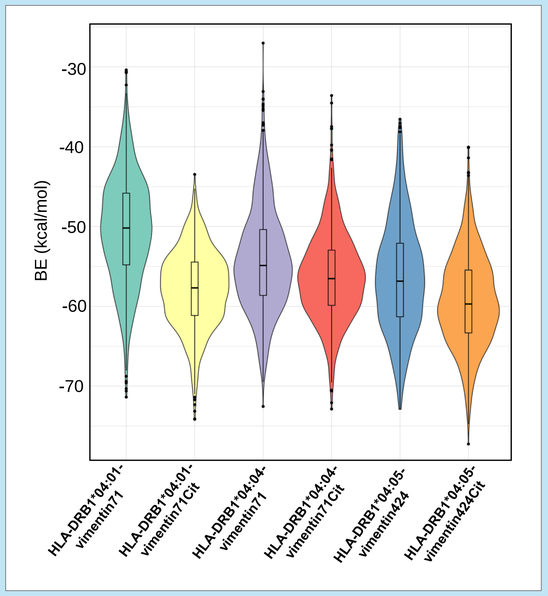

Figure 4: pMHC Binding Escore (BE) calculations for each pMHC system. Binding energies were calculated for 251 pMHC conformations extracted from each trajectory replicate.

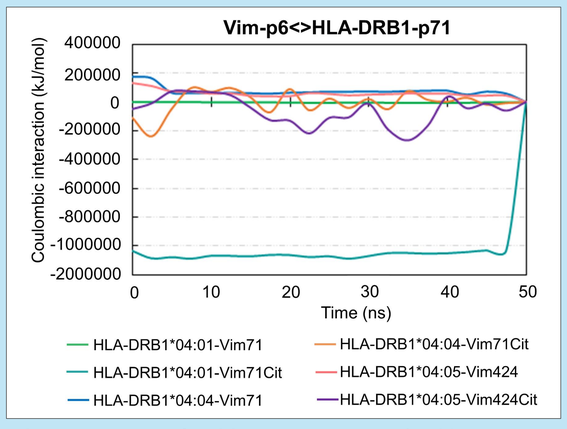

Figure 5: Coulombic energy. Coulombic energy interaction calculated between the amino acid at the position 6 (Vim-p6) of the vimentin and the amino acid at position 71 of the shared epitope in the HLA-DRB1 molecule (HLA-DRB1-p71).

Conclusion

- Citrullination seems to enhance peptide C-terminus stability in HLA-DRB1 complexes, with more prominent in the HLA-DRB1*04:01 molecule (**Figure 2**).
- The pMHC complex with the PTM exhibited an increased number of MHC residues engaged in intermolecular hydrogen bonds during MD simulations (**Figure 3**). Consequently, this enhanced hydrogen bonding contributed to greater stability of the citrullinated pMHC complex compared to the native complex.
- Citrullination has been observed to enhance the binding affinity between peptides and HLA-DRB1 molecules, as compared to their native counterparts **(Figure 4).**
- The presence of citrulline appears to inhibit a repulsive interaction between amino acid 71β within the shared epitope of HLA-DRB1 and the P4 residue of native vimentin **(Figure 5)**.

Then, these points suggest that citrullination may potentially influences the immune response linked to the development of RA.

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REFERENCES:
1. Busch, R., Kollnberger, S., Mellins, E.D. HLA associations in inflammatory arthritis: emerging mechanisms and clinical implications. Nat Rev. Rheumatol. 2019;15(6):364-381; **2**. Scally, S.W., Law, S.C., Ting, Y.T. *et al*. Molecular basis for increased susceptibility of Indigenous North Americans to seropositive rheumatoid arthritis. Ann Rheum Dis. 2017;76(11):1915-1923; **3.** PDB, available at: https://www.rcsb.org/, last accessed in: September 06th, 2023; **4.** CHARMM-GUI, available at: https://www.charmm-gui.org/, last accessed in: September 06th, 2023.