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From a Bioinformatic Approach to Synthetic Conformational Peptide Epitopes to Disclose Molecular Mechanism of Aberrant Glucosylation in Multiple Sclerosis

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Introduction

Multiple Sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system. MS is accepted to be a group of diseases that have not been yet completely characterized.

Autoantibodies (Abs) circulating in blood can be used as specific biomarkers relevant for an early diagnosis or prognosis of a disease form. To this end, a 'chemical reverse approach' based on the use of patient sera to screen focused libraries of synthetic modified peptides, can lead to the identification of selective antigenic probes able to characterize specific and high affinity Abs. MOG (myelin oligodendrocyte glycoprotein) is a glycoprotein present in the CNS (central nervus system) of mammalians and represents 0.01-0.05% of total myelin proteins. Mog possesses two potential transmembrane domains and one *N*-linked glycosylation site on MOG exposed on the outermost surface of myelin making it an ideal target antigen for Ab-recognition. [Asn31(Glc)]-hMOG(30-50) (Table 1) was the first *N*-glucosylated MOG peptide able to recognize specific IgG Abs in sera of a multiple sclerosis patient population (Figure 1) while the unglucosylated analogue hMOG(30-50) was inactive[1].

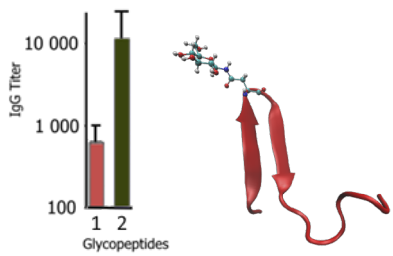

Fig. 1. IgG Ab titers to glycopeptides [Asn31(Glc)]- hMOG(30-50) (1) and CSF114(Glc)(2).

Starting from hMOG(30-50), a structure-activity relationship study allowed to design a scrambled sequence characterized by a β-hairpin structure optimally exposing the minimal epitope Asn(Glc). This peptide termed CSF114(Glc) [2] recognized Abs with higher affinity and greater specificity [3].

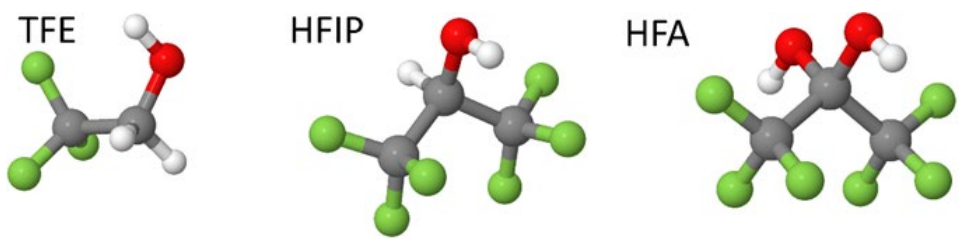

Fig. 2. Molecular structure of TFE, HFIP, and HFA biomimetic solvents.

Table 1. Peptide sequences used in the simulation systems.

Name	*Sequence*
hMOG(30-50)	KNATGMEVGWYRPPFSRVVHL
[Asn31(Glc)]-hMOG(30-50)	KN(Glc)ATGMEVGWYRPPFSRVVHL
CSF114	TPRVERNGHSVFLAPYGWMVK
CSF114(Glc)	TPRVERN(Glc)GHSVFLAPYGWMVK

Results and Discussion

Molecular dynamics (MD) simulations of two N-glucosylated versus the unglucosylated analog peptides were performed to achieve structural properties of different peptide epitopes, using three different mixtures of biomimetic solvents: trifluoroethanol (TFE), hexafluoro-2-propanol (HFIP) and hexafluoroacetone (HFA) (Figure 2). The effectiveness of structure-inducing cosolvent depends on its percentage in the mixture. Mixed water-alcohol solvents (TIP3P model has been adopted for water) [4] may approximate the dielectric constant of protein interiors. In the case of peptides, the folding in these solutions represents an intrinsic propensity to adopt a specific secondary structure in the corresponding protein region, otherwise unstructured in aqueous solution. This solvent mixture is a stabilizing agent, which increases the intrinsic tendency of the amino acid sequence to fold in defined secondary structures. As general trend, HFIP and HFA are considered stronger structural inducers than TFE, suggesting that the ability of the cosolvent is related to the presence of F atoms, and as consequence additional F atoms would be more effective. The lowest energy form of TFE has a gauche configuration, while the trans conformer is less stable, being its energy > 2.0–2.5 kcal/mol [5]. This is in agreement with the hypothesis that TFE molecules form a 50:50 mixture of trans and gauche conformations around the C–C–O–H frame in solution. HFA is known to form stable hydrates. The monohydrate is a solid melting at 46°C, the trihydrate can be distilled and melts at -11°C. Both these hydrates are formed bubbling gaseous HFA into water. If the molar ratio of the components in solution is 1:1, only the monohydrate is formed. Hydrated fluoroketones can be considered as fluoroalcohols, i.e., geminal diols. HFIP has a high tendency to form micelle-like clusters with a maximum effect at about 30% (v/v).

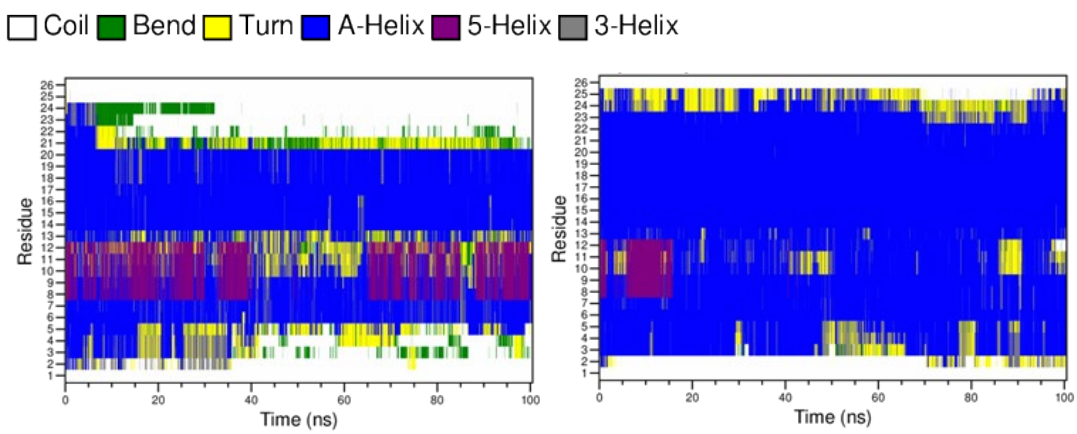

Fig. 3. DSSP analysis of Melittin in HFA with both GAFF (left) and with improved FFs (right).

We report herein, an improved Force Field (FF) for TFE, HFIP, and HFA that use the General Amber Force Field (GAFF) [6], to describe bonded and Lennard-Jones parameters. Atomic charge was reparametrized by using Mulliken population Analysis [7] considering the bulk solvent effects in the Conductor-like Polarizable Continuum Model (C-PCM) [8]. As case study, to verify the accuracy and reliability of the FFs, was chosen Melittin (MLT), a component of the venom of *Apis mellifica*. MLT is a 26-mer peptide that is unstructured in water at low pH but adopts an α-helical conformation when it is bound to the membrane as well as when alcohols are present. MLT consists of two α-helical regions and these portions are connected through Thr11 and Gly12. In Figure 3, we report the (DSSP) define secondary structure of protein analysis showing a more stable structure when the improved FF is used. These FFs have been employed to simulate the structure-based designed type I' β-turn peptide CSF114, hMOG(30-50), CSF114(Glc), and Asn31(Glc)]hMOG(30–50) with the glucosyl moiety added thanks to the tool doGlycans[9].

Previously, NMR-based conformational studies revealed that Asn31(Glc)]hMOG(30–50) and hMOG(30-50) adopted similar conformations in water/HFA (50:50 v/v) solution [10]. The specific autoantibody binding site on hMOG(30-50) was related to the *N*-linked glucose moiety suggesting it as minimal epitope. During simulation of hMOG(30-50) in HFA, the average time development of hydrogen bond distances for i-(i+3) backbone (left) and i-(i+2) CO on Asn side-chain (right), suggest that hMOG(30-50) adopts an Asn-turn (Figure 4). That can explain the lower antibody titer detected in MS sera.

NMR-based conformational studies on CSF114 show the presence of a β-hairpin structure (i.e. a β-turn flanked by two antiparallel β-sheet) in HFA/water 50/50 v:v [11]. DSSP graph (Figure 5) shows the ability of improved FFs reproducing the structure of the designed type I' β-turn peptide CSF114 (Glc).

Future studies will be aimed to correlate aberrant asparagine-glucosylation (N-Glc) and ability to detect autoantibodies in MS patient sera. We will focus on bioinformatics to characterize consensus sequences in myelin proteins in order to identify *de novo* peptides. These *de novo* sequences will be studied by molecular dynamics to verify secondary structures in water and biomimetic solvents. Then the optimized structure will be synthesized and tested for antibody recognition in MS.

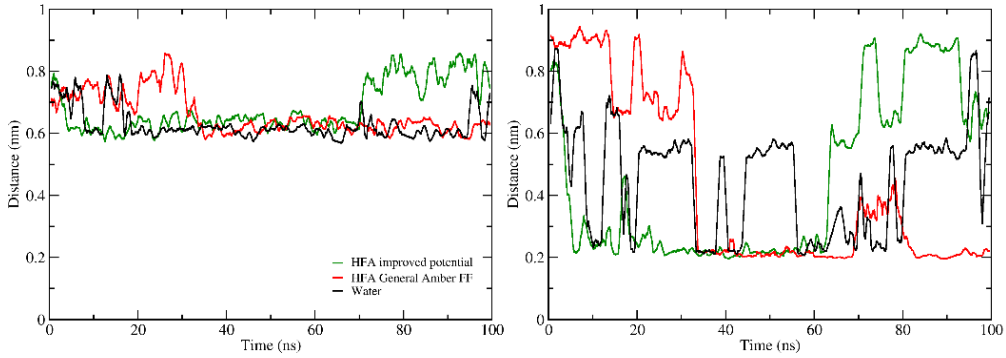

Fig. 4. Average time development of 30Lys-O-33Thr-H hydrogen bond distances (β-turn, left) and 31Asn-CO-33Thr-H (Asn-turn, right) for hMOG(30-50) in HFA and water simulation.

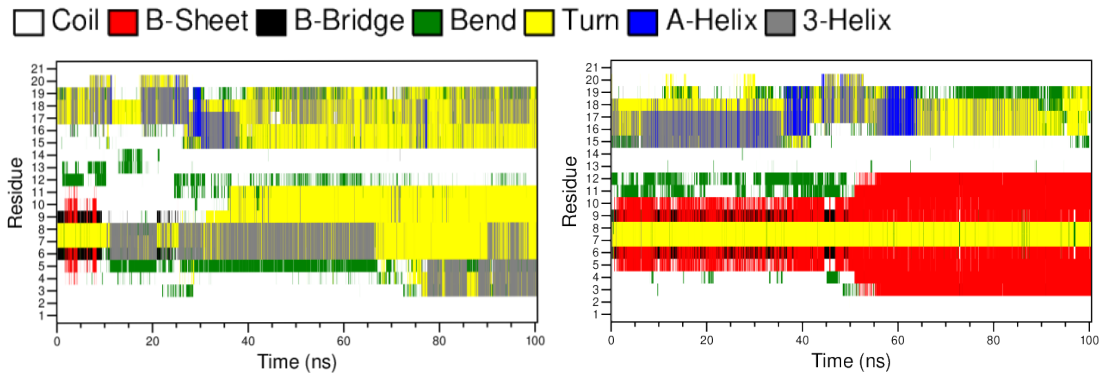

Fig. 5. DSSP graph of CSF114(Glc) in TFE using GAFF (left) and the improved FF (right).

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References

1. Mazzucco, S., et al. *Bioorg. Med. Chem. Lett.* **9**, 167-172 (1999), https://doi.org/10.1016/S0960-894X(98)00698-2
2. Lolli, F., et al. *PNAS* **102**, 10273-10278 (2005), https://doi.org/10.1073/pnas.0503178102
3. Lolli, F., et al. *J. Neuroimmunol*. **167**, 131-137 (2005), https://doi.org/10.1016/j.jneuroim.2005.05.016
4. Jorgensen, W.L., et al. *J. Chem Phys*. **79**, 926-935 (1983), https://doi.org/10.1063/1.445869
5. Li-Hong, X., et al. *J. Chem Phys* **103**, 9541-9548 (1995), https://doi.org/10.1063/1.469968
6. Procacci, P. *J. Chem. Inf. Model* **57**, 1240-1245 (2017), https://doi.org/10.1021/acs.jcim.7b00145
7. Macchiagodena, M., et al. *Chem. Phys. Lett.* **677**, 120-126 (2017), https://doi.org/10.1016/j.cplett.2017.04.004
8. Barone, V., et al. *J. Chem Phys* **102**, 1995-2001 (1998), https://doi.org/10.1063/1.469968
9. Danne, R., et al. *J Chem Inf Model* **57,** 2401-240 (2017), https://doi.org/10.1021/acs.jcim.7b00237
10. Carotenuto, A., et al. *J. Med. Chem*. **44**, 2378-2381 (2001), https://doi.org/10.1021/jm010811t
11. Carotenuto, A., et al. *J. Med. Chem.* **49**, 17 (2006), https://doi.org/10.1021/jm060117j