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In silico investigations of N-glycosylation role in modulating IgG1 conformational behavior and Fc effector functions

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Currently, monoclonal antibodies (mAbs) are the most used biopharmaceuticals for human therapy. One of the key aspects in their development is the control of effector functions mediated by the interaction between fragment crystallizable (Fc) and Fcγ receptors, that is a secondary mechanism of action of biotherapeutics. N-glycosylation at Fc portion can regulate these mechanisms and many experimental evidences suggest that modifications of glycosydic chains can affect the antibody binding to FcγRIIIa, consequently impacting the immune response. In this work, we try to elucidate *via in silico* procedures the structural role exhibited by glycans, particularly fucose, that can potentially affect the receptor recognition. By using adalimumab, a marketed IgG1, as general template, after rebuilding its three-dimensional (3D) structure through homology modeling approaches, we carried out molecular dynamics simulations of three species: aglycosylated, afucosylated and fucosylated antibody, alone and in complex with FcγRIIIa. Trajectory analyses showed different dynamical behaviors among antibodies, highlighting that sugars can influence the overall 3D structure of the molecule and the orientation of Fragment antigen binding (Fab) domains. Moreover, oppositely to what happens in the fucosylated complex, in absence of fucose Fab arms can participate to the receptor recognition and many antibody residues considered critical for the complex formation by mutagenesis studies were found to interact with FcγRIIIa. Our study suggests a putative structural mechanism by which the fucose introduces conformational constraints in the whole antibody and not only in the Fc domain, preventing a conformation suitable for the interaction with the receptor. As secondary evidence, we observed a high flexibility of the antibodies that is translated in an asymmetric behavior of Fab portions shown by all the simulated biopolymers, suggesting a new molecular aspect that may be deeply investigated. In conclusion, these findings can help understand the contribution of sugars on the structural architecture of mAbs, paving the way to novel strategies of pharmaceutical development.