B4 Computational characterization of the CDR regions of two distinct mabs targeting the interaction with HBSAG

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Introduction: Hepatitis B virus (HBV) infection is one of the major health problems worldwide. The diagnosis of the infection is based on the detection of hepatitis B surface antigen (HBsAg) by the use of monoclonal antibodies (mAbs). We report here the investigation of two specific Fv fragments against HBsAg that were developed by the use of hybridoma technology.The sequences of complementarity determining regions (CDRs) of VH and VL regions were determined in order to investigate the main aspects of the mAbs affinity against the antigen of interest based on bioinformatics analysis. The Homology Modeling methodology was used to build the 3D model of Fv, followed by Docking with the antigen. Complexed Fv structures were analyzed by molecular dynamics (MD).

Objective: Computational structural characterization of mAbs aiming at an understanding of the molecular parameters involved in the recognition of HBsAg in order to establish a structureactivity correlation and to improve the affinity of the mAb.

Methodology: In order to build the light and heavy chains of the A and B clones, the Modeller v.9.12 software was used. The template structures were 1JRH and 3LIZ respectively. The docking with HBeAg provided the confirmation of the binding site. The MD simulations were processed to assess the conformational features of both mAbs.

Results: The identity found between the light and heavy chains was 86,5% and 82,9%, respectively. Validation of the homology model was done with Procheck and Verify3D softwares. The values for Procheck are above the minimum values considering the residues in the most favored regions, while according to Verify3D, more than 80% of the residues have a score higher than 0.2. The putative binding site was characterized by molecular docking with Haddock software, allowing the identification of the residues involved in the recognition between HbsAg and the mAbs. According to the MD analysis, the thicker regions in the structure depict the segments with wider flexibility and the thin regions highlight rigid segments of CDRs and suggest functional importance in the molecular recognition. The next steps will be the mutation of specific aminoacids followed by the evaluation of the theoretical free energy and the affinity between the mAbs and HBsAg peptides.

Conclusion: The results showed structural aspects of the Fv fragments for both mAbs that contribute significantly in the affinity interaction with the antigen and will be explained by the analysis of the complex. The flexibility showed by some CDR regions reveals a variety of conformations that enable them to accommodate the antigen. Based on these results, site direct mutagenesis can be suggested in order to improve the affinity between the mAb and the HBsAg surface antigen.

Keywords: Molecular Dynamics, mAbs, HBV, Homology