Modeling of Chimeric β-galactosidase Antigenic Fusion Proteins

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1 Introduction

It has been shown that the insertion of antigenic peptides of the foot-and-mouse disease virus (FMDV) into specific sites of the bacterial enzyme β-galactosidase causes a severe reduction of the activity of this enzyme which can be recovered upon antibody binding to the inserted antigenic peptide [1]. In principle, the recovery of β-galactosidase activity upon antigen-antibody binding allows the detection of antibodies by simple colorimetric quantification of β-galactosidase activity in a homogeneous test system, involving only the antigenic fusion protein, a chromogenic substrate and a serum sample for testing.

The lack of three-dimensional (3D) structural information on the chimeric proteins renders their modeling requisite to understand at the molecular level the chimeric enzyme inactivation and re-activation upon antibody binding. For this purpose, we have determined 3D models of chimeric β-galactosidase by comparative modeling and structure prediction techniques.

2 Methods and Results

Construction of the antigenic peptide inserted at position 278 and 795 of the β-galactosidase enzyme has been performed since these constructs are known to display high antigenicity and reactivation upon antibody binding. To model the chimeric proteins three main steps are performed.

(1) Modeling of the most probable conformations of the FMDV antigenic peptide to be inserted

Several Xray structures of the whole viral protein are available. The only structures however that show a well-defined conformation of the antigenic peptide are that of a mutant virus lacking one disulfide bridge (serotype O) and that of a short peptide (13 aa, serotype C) in interaction with SD6 antibody. Both experimental [2,5] and prediction data [3] agree on the local conformation: the segment before the highly conserved triplet Arg-Gly-Asp, which is involved in the recognition process, is in an extended conformation and the segment beyond adopts a helical conformation. However the two experimental Xray structures show a relative orientation of the two segments that markedly differs. To model the peptide to be inserted (27 aa from serotype C) by comparative modeling [4], only the serotype O structure is used as it displays a peptide sequence long enough even though it presents the drawback of a non-entirely homologous sequence (serotype O versus C).

(2) Modeling of the 3D structures of the peptide-inserted β-galactosidase

β-galactosidase Xray structure, secondary structure predictions on the chimeric sequence and comparative modeling were exploited to produce 3D models of the chimeric proteins. Two- and four-residue lengths are chosen to anchor the antigenic peptide onto the enzyme so as to sample different potential orientations at the enzyme surface. Overall 165 3D dimeric models are generated for each insertion site. A clustering procedure based on the computed root mean square deviation of the backbone atom coordinates is applied to the models. Multi Copy Molecular Dynamics simulations are performed on the representative dimeric model of each cluster to improve the conformational sampling of the inserted fragment accounting for its flexibility. As residues in the vicinity of the insertion at position 278 in the wild-type protein are known to interact with the active site of the facing monomer, conformational analysis of the dimers is performed to detect interactions between the inserted peptide in one monomer with active site residues of the other that could account for partial inactivation of the chimeric protein as experimentally observed. Insertion at position 795 is shown to interact with active site residues within the same monomer.

(3) Modeling the interaction of the chimeric enzyme with an antibody molecule

The inserted fragments that display a solvent accessibility surface area large enough to bind an antibody molecule are selected. Tetramers are visualized to test their ability to bind an antibody molecule. The latter is positioned by fitting the SD6 antibody-antigen complex Xray structure onto the inserted peptide in the 3D models.

The generated models should help, in one hand, to interpret and rationalize the large wealth of experimental data previously obtained. In a second hand they should permit to guide new experiments on inserted-peptide enzymes and more particularly on new recombinant β-galactosidase HIV antigenic fusion proteins as enzymatic probes for anti-HIV antibody detection in HIV infected individuals.

References

[1] Feliu, J. X., Ramirez, E., and Villaverde, A. Distinct mechanisms of antibody-mediated enzymatic reactivation in β-galactosidase molecular sensors, *Febs. Letters* 438:267–271, 1998.

[2] Logan, D., Abu-Ghazaleh, R., Blakemore, W., Curry, S., Jackson, T., King, A., Lea, S., Lewis, R., Newman, J., Parry, N. *et al.*, Structure of a major immunogenic site on foot-and-mouth disease virus, *Nature*, 362(6420):566–568, 1993.

[3] Rooman, M.J., Kocher, J.P. and Wodak, S.J., Prediction of protein backbone conformation based on seven structure assignments. Influence of local interactions, *J Mol Biol.*, 221(3):961–979, 1991.

[4] Sali, A. and Blundell, T.L., Comparative protein modelling by satisfaction of spatial restraints, *J. Mol. Biol.*, 234(3):779–815, 1993.

[5] Vergaguer, N., Mateu, M.G., Andreu, D., Giralt, E., Domingo, E., and Fita, I., Structure of the major antigenic loop of foot-and-mouth disease virus complexed with a neutralizing antibody: direct involvement of the Arg-Gly-Asp motif in the interaction, *Embo J.*, 14:1690–1696, 1995.