Journal of Immunological Methods 425 (2015) 51–57

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

A computational study of the interaction of the foot and mouth disease virus VP1 with monoclonal antibodies

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ARTICLE INFO

Article history:
Received 16 September 2014
Received in revised form 16 March 2015
Accepted 9 June 2015
Available online 17 June 2015

Keywords:
FMDV
Interaction energy
Epitope
Antibody
ELISA
Bioinformatics

ABSTRACT

Foot and mouth disease is caused by a non-enveloped virus (FMDV), which disposes several antigenic sites at the surface of their capsid proteins. The most relevant and immunodominant antigenic site of FMDV (site A or AnSA) includes a key virus–cell interaction element (RGD motif) located in the Viral Protein 1 (VP1), more precisely at the GH loop. AnSA includes a set of overlapped and mainly linear epitopes, which are the main targets of the humoral immune response. Taking advantage over specific structural features of the GH loop, we have evaluated the influence of every amino acid residue at AnSA in the interaction with 2 neutralizing antibodies by molecular modeling techniques. Additionally, we constructed diverse interaction complexes with multiple site A mutants and discussed about the structural influence of amino acidic insertions in such relevant antigenic site of FMDV. Our approach is in agreement with previous ELISA experiments and allows the understanding of how FMDV mutations may alter the interaction with different antibodies, as we can estimate the contribution of each amino acid to the interaction. Overall, our work contributes to the development of specific vaccination strategies for FMD control.

1. Introduction

Foot and mouth disease virus (FMDV) is the etiologic agent of one of the most contagious disease in farm animals. The mechanisms by which the host neutralizes the viral infection lies in a molecular recognizing phenomenon: antibodies (Ab) interact with (and physically occludes) several capsid regions indispensable for host–receptor interaction and viral entrance (Surovoi et al., 1988; Fry et al., 1999). Consequently, worldwide health policies established preventive vaccination as a top FMD control strategy, which implies the development of an antibody-based immune response at a molecular level (Doel, 2003; Sutmoller et al., 2003).

The non-enveloped capsid of FMDV (a picornavirus) is formed by three major proteins that present a great percentage of amino acidic homology and a common fold with a jelly roll topology (Fig. 1 of Supplemental data) (Rueckert, 1996). The main sequence and conformational dissimilarities reside at loops that protrude out of the viral surface and expose relevant neutralizing antigens (Ag). The GH loop (which connects the G and H beta sheets) of the VP1 contains the viral immunodominant or the principal antigenic site (AnSA). AnSA contains an amino acidic stretch highly conserved over the genus: the virus/cell interaction motif Arg–Gly–Asp (RGD) (Jackson et al., 2003; Mateu and Verdaguer, 2004).

The antigenic properties of the capsid proteins have been studied by diverse biochemical, immunological, spectroscopic as well as crystallographic and cryo-electron microscopy (cryo-EM) approaches. In fact, the individual contribution of every amino acidic position at the AnSA of the FMDV of serotype Chas been exhaustively explored in its interaction with seven monoclonal antibodies (mAbs) (Verdaguer et al., 1998). This research also details crystallographic determinations for complexes of 4C4 or SD6 mAbs with AnSA peptides and thus reveals important conserved structural characteristics (Verdaguer et al., 1998; Ochoa et al., 2000). In their study, the authors demonstrated that, upon binding to the viral peptide, both 4C4 and SD6 mAbs underwent considerable structural rearrangements, reaching a similar pattern of interactions. In those complexes, two residues were structurally conserved, Asp143 and Leu144, which were part of the cell receptor recognition motif (Jackson et al., 2003).

In other words, scientific evidence suggests that the interplay between viral and host evolution forces during the host immune response induced by AnSA of FMDV of serotype C produce a repertoire of antibodies which were structural biased because of the proper nature of the antigen: its immunodominance. In this sense, a tool that enables to rationalize and

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http://dx.doi.org/10.1016/j.jim.2015.06.008
0022-1759/

quantify how changes in amino acid composition, both in the FMDV or in the antibodies, modify protein–protein interactions could be invaluable to understand how FMDV escapes antibody-based immune response. Furthermore, it could be useful for the design of novel proteins to recognize FMDV.

The present study uses a bioinformatics approach to model the referred interactions between AnSA of FMDV of serotype C and mAbs. We have critically contrasted our implementation and results with the diverse wet-lab and bench interaction data previously published (Verdaguer et al., 1998). We use the program FoldX (Schymkowitz et al., 2005; Kiel and Serrano, 2007) and Modeller (Sali and Blundell, 1993) to model mutations and analyze the energetic contribution.

Specifically, we developed more than 200 Ag–Ab complexes with point mutations at the Ag sequence and calculated their ΔG (free energy of unfolding). The obtained interaction data effectively pointed those amino acidic positions at the AnSA that were relevant to the Ag/Ab interaction. The same protocol was applied in a prediction approach of the interaction of seventeen viral peptides carrying multiple amino acid mutations (resembling field and laboratory FMDV strains) and comparing the obtained ΔG values with reference interaction ELISA assays.

The developed protocol not only allows one to understand about how each amino acid change affects FMDV recognition by antibodies at the atomic level but it enables the quantification in terms of free energy contribution. Overall, our protocol could be extended to other antibodies or proteins that interact with FMDV AnSA peptides and contributes to the design of novel proteins to recognize FMDV.

2. Materials and methods

2.1. Viruses and mAbs

FMDV antibody neutralization at the immunodominant AnSA has been well described by Ochoa et al. (2000), who provide the only crystallographic determination (PDB1ejo) with proper atomic resolution. Specifically, 1ejo describes an interaction complex between the peptide sc30 (AnSA of FMDV strain sc30) and a mAb (named 4c4). Antibody sequences (4c4 and sd6, see Fig. 2 of Supplemental Data) were obtained from Mateu et al. (1990).

The interaction complex for the close FMDV strain sc8c1 was obtained by in silico mutation of the sc30 AnSA (see below) to the amino acidic sequence of sc8c1 AnSA (named PDB 1ejo81). Comparative modeling with the Modeller program was used when there were missing amino acids in the X-ray structures (Sali and Blundell, 1993) or when shorter/longer peptides were reported as compared to the X-ray structures.

The reference experimental data for computational experiments using different FMDV strains (multiple mutants) were obtained from Feigelstock et al. (1996) and Mateu et al. (1987, 1990) (see Fig. 3).

2.2. Single mutant development and interaction energy calculation

Starting from 1ejo (Ag sc30/mAb 4c4) and 1ejo81 (Ag sc8c1/mAb 4c4), we used the FoldX software (Schymkowitz et al., 2005) for modeling complexes with punctual mutations of the AnSA. Mutations were conducted by systematic single-residue replacement at every position with the 20 genetically coded amino acids.

FoldX was also used to model complexes with the sd6 mAb (Ag sc30/mAb sd6, Ag sc8c1/mAb sd6). The referred software bases its algorithms on an empirical force field definition to provide a fast and a quantitative estimation of the importance of the interactions contributing to the stability of protein complexes (Kiel et al., 2004; Schymkowitz et al., 2005). The software not only yields a mutant complex but also a mutant-specific WT complex. Both are useful to obtain the absolutes energetic values of Ag–Ab interaction, as well as the variation between the mutant and WT the free energy of unfolding at the interaction interface (ΔG).

The FoldX routines were implemented through scripts described in the software manual. Specifically, the scripts for the complex interface repairing (pre-process of the PDB files), mutant building and energy determination were *RepairPDB*, *BuildModel* and *AnalyseComplex* respectively. These scripts are available at the program web page (http://foldx.crg.es/).

2.3. Development of multiple mutant peptides

Viral antigens with multiple mutations (see sequence in Fig. 3) were obtained modifying the basic protocol implemented for single mutations. We built a new template that has a poly-alanine peptide built in the base of the original 1ejo backbone as the AnSA. Such procedure, concerning the usage of poly-ala template, would promote a normalized scenario to build better interaction complexes with a less biased multiple mutated antigens.

Full mutants were also evaluated by making a model with the program Modeller, which allows reorganization of the backbone. Free energy of the interactions was also evaluated with the FoldX program with *AnalyseComplex*.

2.4. Data processing

Re-coding approach was based on the fact that information of any ELISA experiment reflects a relation of masses in the interaction phenomena. In that sense, the IC 50 value relates concentration of mutant peptide which causes 50% of inhibition of the binding between WT peptide and a given antibody.

We calculated a binding constant k from the Gibbs free energy from reference ELISA IC 50 experiments (Verdaguer et al., 1998), and defined the boundaries for the 4 interaction categories in our dataset of interaction energies (Table 1). Specifically, the k related the interaction between a non-saturating amount of a given mAb and 5 pmol of WT peptide that is inhibited by increased amounts of mutant peptides (5, 25, 125, and 625 pmol) (Verdaguer et al., 1998). Subsequently, each computational energetic value (ΔG) was assigned to one of the four interaction categories (Figs. 3 and 4 of Supplemental Data).

ZT software was used to conduct mantel test and statistical analysis (Bonnet and Van De Peer, 2002).

Chimera software and inner plugins were employed in visualization of structures, structural alignments, contact overlap, charge distribution and surface calculations (Sanner et al., 1996; Pettersen et al., 2004).

3. Results and discussion

The present research explored computationally structural features of relevant Ag–Ab interactions in the cluster of serotype C. The Novo Ag–Ab complexes with single and multiple mutations were built by single and multiple-state approach, evaluated and compared with reference data coming from diverse referential research scenarios and ELISA experiments.

3.1. Computational characterization of FMDV AnSA/mAb interaction

We have assessed by two computational approaches the impact of all possible point amino acidic substitutions at every residue of a peptide sc8c1 (representative of the viral AnSA), and its influence in the

Table 1
ELISA categories expressed in ΔG ranges.

ELISA category	ΔG range
1	<0.96
2	0.96–2.04
3	2.04–2.76
4	>2.76