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Research paper

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

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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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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

interaction with mAbs (at Supplemental Data, Fig. 3; mAb 4c4, Fig. 4; mAb sd6). Firstly, we used FoldX software to measure the variation of the free energy of unfolding at the interaction interface (ΔG). The energetic values were then converted to a four grade interaction scale (as explained in the Material and methods section). These computational data when compared with interaction data obtained by ELISA, showed a non-weak Pearson correlation (r) of 0.7, with a high significant level of confidence (simple Mantel test) (Bonnet and Van De Peer, 2002).

In a close view, the pattern of tolerance to mutations in the computational matrixes reveals which amino acidic positions of the AnSA, which in coincidence with ELISA matrixes, were sensitive or relevant. For example, in our approach, the amino acids Gly 142 and Asp 143 tolerate none or few amino acidic changes. Interestingly, both amino acids are part of the RGD motif and have been shown to be important interacting elements displaying a low level of spontaneous mutation in nature (Verdaguer et al., 1998; Ochoa et al., 2000). Fig. 1 illustrates complexes for mAbs 4c4 and sd6 with peptide sc8c1 where precisely the residue Asp 143 was mutated for a Glutamate (a chemical equivalent residue). It can be seen that this small change produces atomic clashes with Arginine 98 at the H-CDR3 in both mAbs, and therefore makes the interaction less favorable. Interestingly, Arg 98 residue and its interactions with viral Asp 143 was pointed by other authors as one of the most relevant in the interface between peptide sc8c1 and mAbs (Verdaguer et al., 1994, 1995, 1996, 1998).

The same increase of the ΔG values was observed for amino acid changes at the C-terminal of the AnSA (residues 144, 146 and 147), which is an antigenically relevant region that is also involved in the virus/host cells interactions. In the referred tolerance pattern, spacer residues (140, 141, and 145) between regions of relevance were also detected by both matrixes.

As FoldX in its implementation doesn't move the backbone, to circumvent this and to obtain a more dynamic description, we conducted an in silico experiment with a "multistate" foundation, which meant a representations of the interaction complexes by molecular ensembles more than single state or rigid structures. We have determined and compared the "tolerated sequence space" for booth Ag/Ab complexes – the set of sequences that a given protein interface can tolerate while still preserving its function at a defined level – using Rosetta backrub flexible backbone design implemented in the RosettaBackrub server (RBS) (https://kortemmelab.ucsf.edu/backrub/cgi-bin/rosettaweb.py) (Smith and Kortemme, 2008, 2011; Lauck et al., 2010). Fig. 2C, shows such comparison, where for both Ag/Ab complexes, the observed sequence of the antigen falls within the five top-ranked tolerated sequence for each position. Additionally, we implemented a comparison between FoldX, ELISA and RBS datasets. In details, we used a mixed approach that takes as INPUT the RBS ensembles (Fig. 2B) and used the FoldX protocols to build and explored the influence of single mutants in the interaction energy. In a comparison of the matrixes emerged from FoldX or RBS inputs, we have obtain a similar "sequence tolerance landscape" (Fig. 2A). Concerning these, the data – at Fig. 2A – for every point of the curves means an average of the interaction energy for all mutations per residue at data matrices (dataset for ELISA and FoldX graph; Figs. 3 and 4 respectively, dataset for BRS were not included).

The most prominent differences between the curves appear surrounding the residue histidine 146; an amino-acid which is usually elusive in computationally predictions due to subjects as protonation states and rotamer assignation (Smith and Kortemme, 2010; Kim et al., 2013; Petukh et al., 2013).

3.2. Prediction of interaction between mAbs and AnSA with multiple mutations

We then studied the interaction between mAbs and viral antigens with multiples mutations. Mutant peptides were de novo modeled

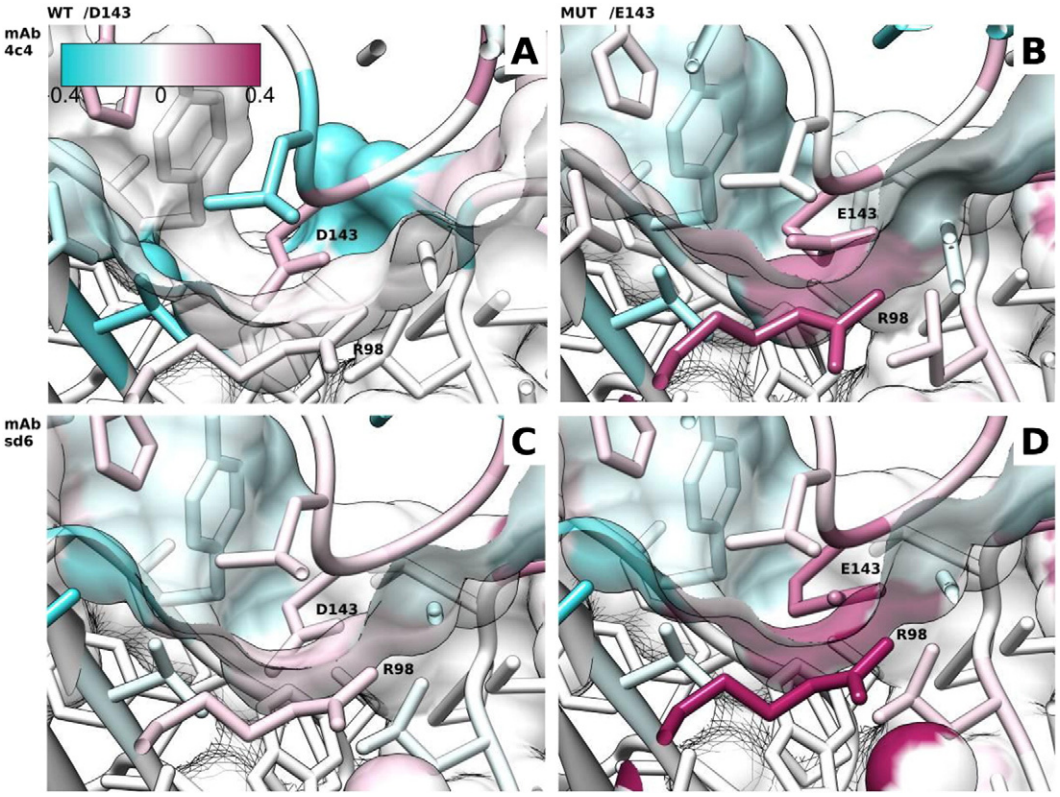

Fig. 1. Influence of point mutation at RGD motif. Mutation of the viral peptide sc8c1 (ASP 143 residue for GLU) in complex with mAb 4c4 (A, B) and sd6 (C, D) (color key: cyan > maroon, low > high atomic overlap). Note the augment of the atomic contacts (clashes) of the ARG 98 and GLU 143 residues in both mutant complexes (B, D), originated by the epitope mutation D143E.

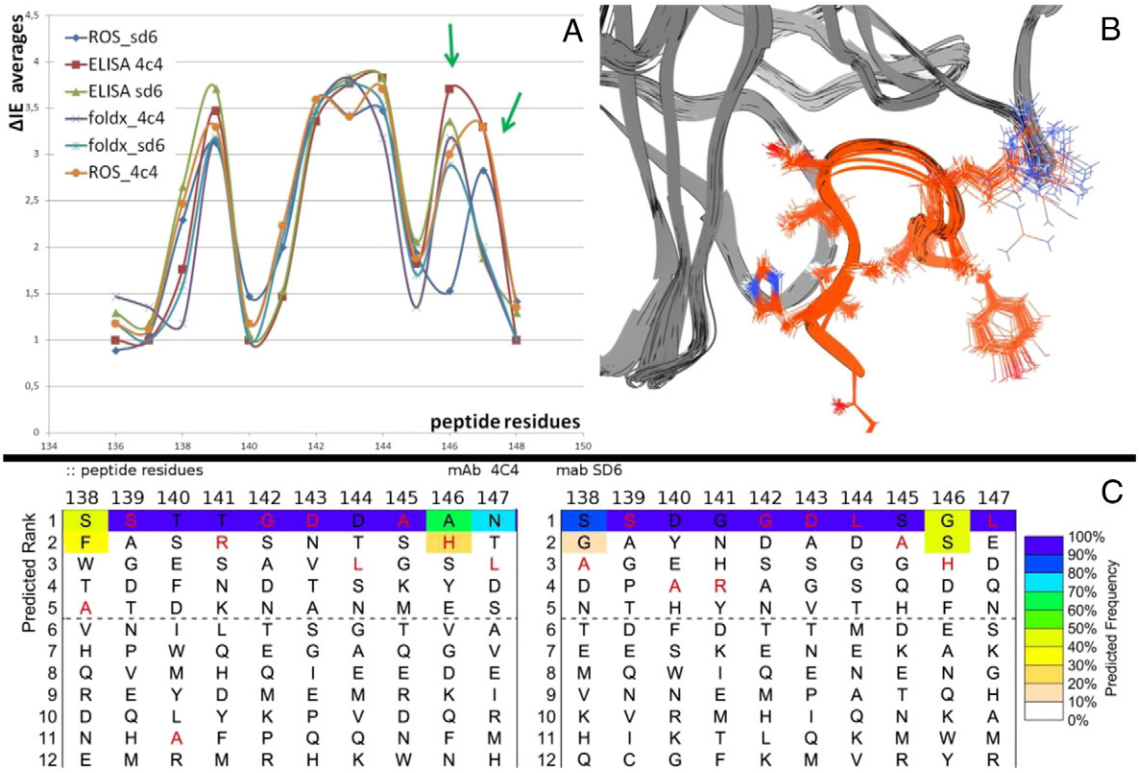

Fig. 2. Single mutant: Comparison between FoldX, Rosetta-based and ELISA Ag–Ab interaction data. A; Each curve links the average ΔG for every residue at the Ag peptide interacting with 2 mAbs and gauged by 3 methods (the arrows indicate the most divergent positions). B; Example of a RosettaBackrub server (RBS) ensemble, used as input to obtain data at Fig. 1A (ROS_4c4). C; Comparison of the tolerated sequence space for interactions between 1 antigen and 2 mAbs, as result from RosettaBackrub server (RBS) (https://kortemmelab.ucsf.edu/backrub/cgi-bin/rosettaweb.py).

from a poli-alanine template of AnSA, as explained in the Materials and methods section, by using the FoldX program.

The referred interactions included the same mAbs sd6 and 4c4, and conformed sixteen complexes in combination with eight AnSA peptides (whose sequence was extracted from field isolates and laboratory strains of serotype C) (Fig. 3) (Mateu et al., 1987, 1990; Feigelstock et al., 1996). The interaction results by ELISA methods are shown in a color code along with the comparisons with the FoldX free energy determinations for the same Ag–Ab complexes (Fig. 3A–E).

We also calculated the free energy contribution of residues at H and L chains of mAbs to the overall interaction energy (ΔG/complex). In a general view, lower energy complexes in the ΔG/H-chain graph were those complexes with better interaction in ΔG/complex graph. The opposite can be appreciated at ΔG/L-chain and ΔG/general graphs sharing the same complexes with higher ΔG. These concordances or clustering of data may be pointing the influence of the H chain residues in positive interactions (complex 1–6) (Verdaguer et al., 1996, 1997, 1998, 1999). Likewise, the augment of general ΔG in negative interactions would be determined by detrimental interactions established by residues at L chains (complex 12–16). In this sense, there is a cooperative effect as the H chain has positive interactions and the L chain regulates variability by not allowing residues to chain, despite the fact that they can interact with the H chain.

Higher dispersion values of ΔG were mainly determined by interatomic clashes of some replicas, an event less frequent in positive interactions complexes. Then, FoldX could be yielding less conformational variable replicas (less clashes) of the certain Ag–Ab complex (which means a recurrent interaction solution) in a case of a positive interaction. Full mutants were also evaluated by using the program Modeller. Through this approach, we were able to reorganize the backbone, and subsequently make an evaluation by the FoldX algorithm. However, no good correlation was obtained. In this sense, we conclude that the backbone interactions are key for protein recognition. When a mutation is incorporated in a protein and the backbone is kept rigid clashes may appear as it happen in several cases for positive ΔGs. However, when the backbone is able to move, as when we used comparative modeling, the clashing problem is overcome by changing the position of the peptide backbone. The fact that we did not find a good correlation with experiments with this approach, points out that backbone reorganization greatly impair binding.

In consequence, we also build a similar set of multiple mutants and its interaction complexes but using the Rosetta backrub flexible backbone approach (including new information from the PDB entry 1QGC) previously optimized by a high-resolution peptide docking (refinement) protocol, implemented within the Rosetta framework (Rosetta FlexPepDock) (London et al., 2011). Fig. 3F resumes the results of the ensemble based implementations; note that a complex as c-7, not so well predicted by FoldX, improves its prediction by RBS protocols. Conversely, complex c-11 with positive Ab interaction by ELISA, was doesn't correctly modeled and predicted by neither of the current protocols.

3.3. Viral mutations that challenges antibody recognition

As it was demonstrated by ELISA (Mateu et al., 1987, 1990; Feigelstock et al., 1996) or by a computational approach, at the present study, mAbs 4c4 and sd6 yielded a very similar pattern of reactivity against a set of viral antigenic variants, even when they display different amino acidic sequences in their CD3 variable domain. The reason of such feature resides mainly at their homologous tertiary structure when they form a complex with a viral peptide.

We also found some exceptions of the previous observation. Natural variants interact with mAbs 4c4 and sd6 in a divergent way. For example, viral peptides C2 UR/45 (complexes 6 and 9) and C4 ARG/66 (complexes 5 and 14, Fig. 3D), which display an arginine insertion before the

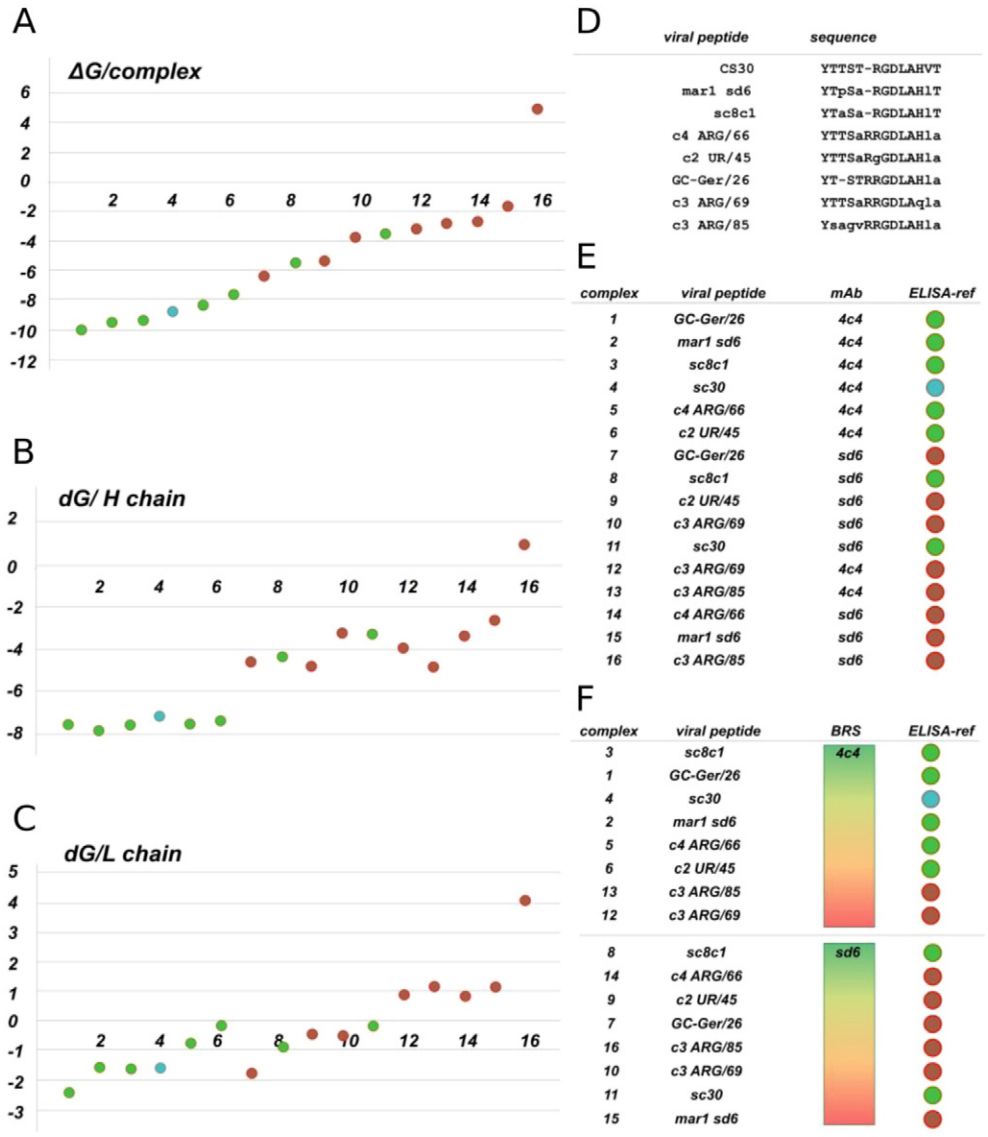

Fig. 3. Multiple mutants: Comparison between FoldX, Rosetta-based and ELISA Ag–Ab interaction data. A; FoldX ΔG (general) for Ag/Ab complexes. B, C; heavy and light chain ΔG (specific). D; Aminoacidic sequence of the Ag (AnSA) peptides. E; Identity of Ag/Ab complexes for each biological sample and its reference ELISA (blue, 1EJO PDB complex; red and green, negative and positive interaction, respectively). F; Ag/Ab complexes order by increasing values of ΔG calculated at BRS (column BRS: gradient from green to red; from less to more ΔG).

RGD motif behave differently. This arginine insertion, in the case of complexes with mAb sd6 (eg. complex 14; Figs. 3 and 4D), results in a worsening of ΔG. On the other hand, some 4c4 complexes establish an alternative net of interactions in the new topology (eg. complex 5 and 6, see Fig. 3B, C); which results in a "conservation" of positive interaction status. That is the case of differential interactions taking place between viral Arg 140 and residue 32 at the L chain of both mAbs. Specifically, at position 32, sd6 mAb presents a serine residue which could be less effective than the Tyr residue (presented by mAb 4c4) in their stabilizing effect over Arg 140. Arginine has a polar character and a long and flexible side chain, which means, in a correct context, a great ionic interaction partner. Indeed, the RGD motif at its context was not trivial. Then, the Arg 140 residue (and its flexible polarity) in a non-favorable topology would became a disturbing force, even more if it is followed by a second Arg at 141 position (integral part of the RGD motif).

Other kind of mutations at AnSA exceeds the paratope's limits of interaction, even for the mAb 4c4, with a wider recognition spectrum. The complexes 10 and 12 (Fig. 4) include an arginine insertion before the RGD motif but they also hold a substitution of histidine for glutamine at position 146. The last mutation does not conserve the polar or the aromatic character of the histidine; which has a direct impact in relevant interaction with aromatics residues at H-CDR of both mAbs.

4. Conclusions

Nowadays, FMDV spreading has been controlled in many countries by preventive vaccination which works by triggering a host antibody-based immune response. However, classical whole-virus inactivated recombinant vaccines have to overcome a viral challenge imposed by the high antigenic variability displayed at different sites over FMDV capsid.

In nature, the viral RGD motif that targets the cellular receptor also exists in host proteins. Both viral and host RGD motif are consequently included in the self-tolerance program of the immune system as a self-antigen. In this concern, the "anti-site A" antibody response seems to be structurally biased by interacting or accessing the site A antigens in a limited conformational space.

We presented a computational approach and analyzed in detail all amino acid residues present at the interaction interface between the viral strain sc8c1 and two neutralizing monoclonal antibodies. The

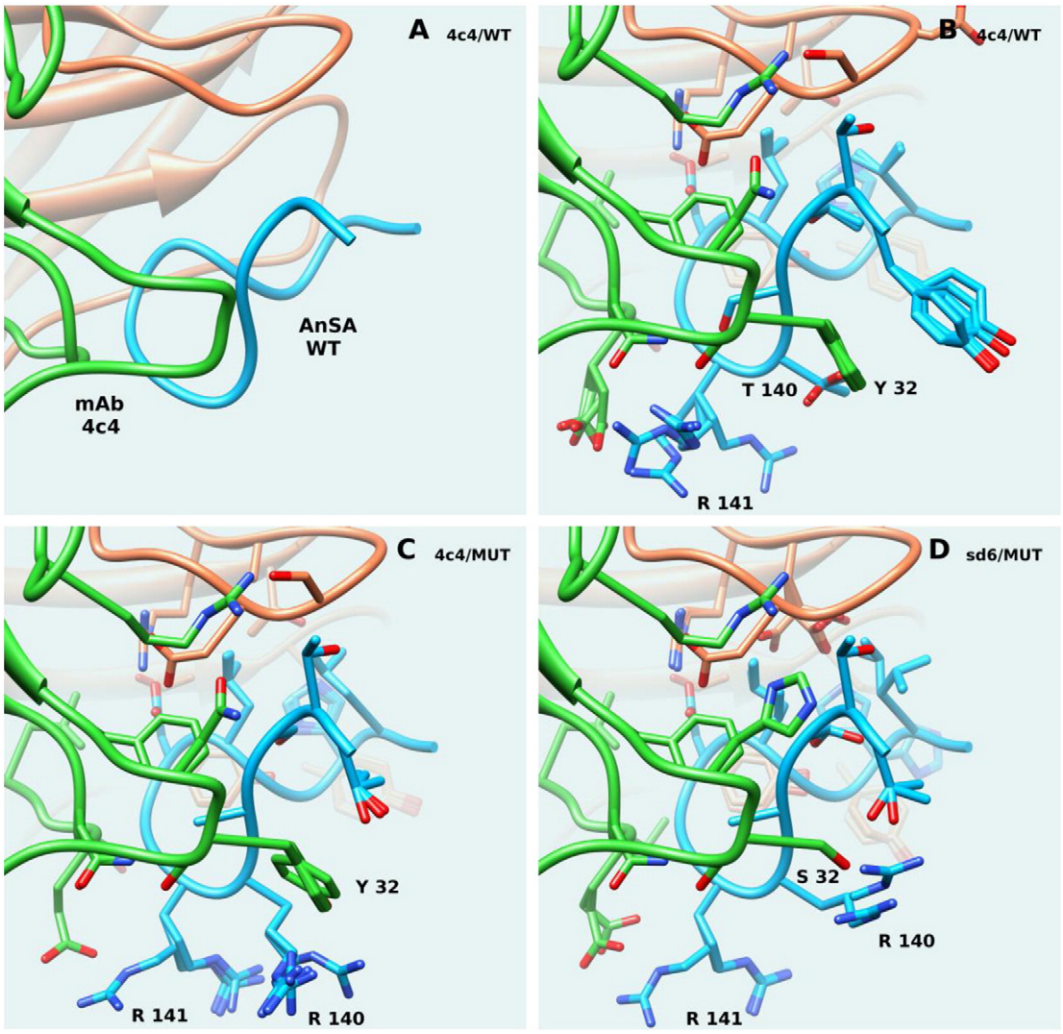

Fig. 4. Influence of the natural occurring mutation near RGD motif. (A) General view of the interaction, H (orange) and L (green) chains, AnSA peptide in light blue. Ribbon diagram of some complexes of Fig. 3: B, complex number 3, mAb 4c4 and AnSA WT (without insertion). C and D, complexes 5 and 14, mutant AnSA with arginine insertion at 140 interacting with 4c4 and sd6 mAb respectively. Note 4c4 mAb at complex C, displaying a tyrosine residue at position 32, occupied in sd6 mAb complex (D) with a serine residue as well as the influence of both residues over arginine at 140 and 141 on AnSA.

results support the previous knowledge and enable to extend the description of the FMDV interaction phenomena.

This work explores de novo Ag–Ab interaction predictions including field isolates and laboratory mutant FMDVs with results that support the usage of computational methods for interaction prediction routines that involve multiple mutant peptides. Undoubtedly, the experiments yielded some inconsistencies that point out that new crystallographic determinations are required to bring better in silico representations of the biological phenomena and the emerging prediction performance of our protocol (Kiel et al., 2004, 2005). Also, there is a clear need for more benchmark data from homogeneous experimental interaction determinations (ELISA) performed on the same experimental framework.

Nonetheless, the methodology described in this study offers new possibilities in the structural characterization of FMDV–mAbs interaction. Its major advantage resides in the achievement of consistent computational prediction assays for a wide number of interactions with a reasonable balance between fastness and effectiveness.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jim.2015.06.008.

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