Converging antigenic structure of a recombinant viral peptide displayed on different frameworks of carrier proteins

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Abstract A peptide reproducing the G-H loop amino acid sequence of foot-and-mouth disease virus VP1 protein was fused to the solvent-exposed C-terminus of the bacteriophage P22 tailspike protein [Carbonell and Villaverde (1996) Gene, in press], a homotrimeric polypeptide with a strong β-helical structure. This fusion does not interfere with the biological activities of the phage tail. The antigenic profile of the complex antigenic site A within the G-H loop has been determined by competitive ELISA with a panel of monoclonal antibodies directed against different overlapping B-cell epitopes. The antigenic data have been compared with those obtained with a set of 12 chimeric β-galactosidases displaying the G-H loop on different exposed regions. A high coincidence has been evidenced between the antigenicity of the viral peptide fused to the phage protein and that of some peptides inserted in an exposed loop of the activating interface of β-galactosidase. This indicates that completely different structural frameworks of carrier proteins can provide similar constraints that allow the recombinant peptide to successfully mimic the antigenicity, and probably conformational features, of the natural peptide on the virion surface.

Key words: Antigenicity; Recombinant protein; Competitive ELISA; Peptide display; Site A; Foot-and-mouth disease virus

1. Introduction

The antigenic site A of foot-and-mouth disease virus (FMDV) is a small peptide of VP1 protein of about 15 amino acids in length and one of the major antigenic determinants of FMDV [1]. In serotype C, this segment contains several continuous, overlapping B-cell epitopes [2] and is located in the disordered, extremely flexible G-H loop which protrudes from the outer capsid surface around the five-fold axis of the eicosahedral structure [3,4]. This peptide also includes the arginine-glycine-aspartic acid (RGD) motif involved in cell recognition and attachment [5,6]. Critical residues in these epitopes have been identified by antigenic analysis of synthetic peptides and by sequencing both monoclonal antibody-resistant (MAR) mutants and field isolates [2,7,8]. On the other hand, amino acid substitutions within this region occur very frequently, thus defining a hypervariable segment that has been used as a model to study features of viral evolution in both laboratory and field conditions [9–11].

The particular complexity of this antigenic site seems to have a structural basis and this fact could be related to the extreme flexibility of the G-H loop, which makes it invisible in crystallographic studies. In fact, it has been proposed that, like a peptide in solution, this loop could adopt a set of conformations against which different monoclonal antibodies are directed [12]. In agreement with this suggestion, the antigenicity of a recombinant G-H loop sequence displayed on a chimeric protein is better than that of a lineal peptide and it varies upon the specific insertion site [13]. This indicates that the framework provided by a carrier protein can create structural constrictions that modulate the antigenic mimicry. This observation could be of great interest regarding the design of recombinant peptides that conserve their natural activities.

In this connection, we have explored the antigenic profile of the FMDV G-H loop when displayed at the C-terminus of the homotrimeric tailspike protein (TSP) of *Salmonella* bacteriophage P22 [14], and compared this pattern with that of the same peptide presented on different regions of the β-galactosidase surface. Interestingly, the chimeric TSP is highly antigenic and the viral peptide shows an antigenic profile similar to that observed on the virus surface, but especially to one among the chimeric β-galactosidases. This converging antigenic profile indicates that more than one particular framework can provide the structural requirements for an improved antigenic mimicry of a peptide inserted in a recombinant, carrier protein.

2. Material and methods

2.1. Recombinant proteins and other antigens

TSPA is a TSP in which the FMDV G-H loop peptide from isolate C-S8c1 (amino acids 134–156) has been fused at its C-terminus [15]. This protein has been produced from a pTrc99A (Pharmacia) derivative by IPTG-mediated induction in *E. coli* BL26. Chimeric P22 particles were obtained by in vitro association as described [15]. Most chimeric β-galactosidases have been previously described [16,17]. All of them contain the complete antigenic site A sequence (amino acids 134–156 of protein VP1) inserted in different regions of the recombinant enzyme. AB1 is an N-terminal fusion of a larger VP1 region including the 134–156 peptide [13]. AB1278VP1 is a hybrid between AB1 and M278VP1 proteins constructed as described for AB1275VP1 [17]. The chimeric β-galactosidases were produced in MC1061 *E. coli* strain from pJLA602 derivatives by heat induction. The concentration of the viral peptide in cell extracts was calculated by Western blot as described [13]. UV-inactivated FMDV particles and peptide A21 were generously provided by M.G. Mateu and D. Andreu, respectively.

2.2. Competitive ELISA and monoclonal antibodies

Monoclonal antibodies raised against the whole FMDV particles but directed to site A have been previously described [2,8]. Competitive ELISA was performed according to a previously described protocol [18]. Peptide A21 reproduces a segment of G-H loop containing FMDV site A [10,13]. This peptide, coupled to KLH (A21-KLH), was used as a coating antigen for microtiter plates. 5 pmol of peptide per well was plated and incubated overnight at 4°C in carbonate buffer. ELISA plates were blocked with 5% BSA in PBS for at least 2 h and

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then washed with PBS. 95 μl of a solution containing non-saturating, fixed amounts of mAbs and different amounts of antigen (2, 6, 18, 54, 162 and 486 nM) in 1% BSA were plated and further incubated at room temperature for 1 h. Non-bound antibodies were removed by washing with PBS–0.05% Tween. Bound mAbs were detected by using peroxidase-conjugated goat anti-mouse IgG (Bio-Rad) (100 μl of a 1:3000 dilution) and developed with 4-chloro-1-naphthol and H_2O_2 as substrates. The reaction was stopped with 50 μl of H_2SO_4 2 M and the absorbance read at 620 nm.

3. Results and discussion

3.1. Antigenicity of the FMDV peptide fused to the P22 TSP

In a previous work, it has been shown that the FMDV G-H loop peptide, fused to the C-terminus of P22 TSP, is solvent-exposed and accessible to antibodies [15]. The antigenicity of soluble, homotrimeric TSPA protein was studied using a set of seven mAbs elicited against the whole virus particles but directed to different epitopes within site A. In Fig. 1A are depicted the results obtained in an ELISA in which TSPA competed with A21-KLH peptide bound to plastic wells. The chimeric protein is recognized by all the mAbs with different efficiencies whereas TSP is not recognized at all. In particular, SD6 and SB10, which are closely related and probably recognize the same B-cell epitope [2,18,19], show a poor immunoreactivity with TSPA, while the rest of the antibodies recognize the antigen better. Since the panel of mAbs used is representative of the average reactivity of sera from infected animals [13], this result suggests that the majority of the viral B-cell epitopes contained in site A are efficiently reproduced in the chimeric TSPA. Fig. 1B shows the reactivity of two mAbs with TSPA after in vivo association with tailless P22 heads. No significant differences of reactivity are evident between head-associated and free TSPA, indicating that the interaction of the N-terminus of TSPA to the virus heads does not produce alterations in the opposite C-terminus that could modify the solvent presentation of the antigenic site. Concentrations of competitor antigen leading to 50% inhibition of the maximum reactivity (IC_{50}) were calculated for all the mAbs.

3.2. Antigenic analysis of chimeric β-galactosidases

Competitive ELISAs were performed to determine the antigenicity of 12 chimeric β-galactosidases displaying FMDV site A on different insertion sites. In addition, whole virus particles and a synthetic peptide reproducing site A were also studied. The obtained results allowed us to calculate, for each protein competing with bound A21-KLH peptide, an IC_{50} value (the molar amount of antigen necessary to reduce reactivity in ELISA to 50%). These values, similar to those obtained when competing with the whole virus [13], were compared with those presented in Fig. 1A for TSPA. For each antibody and protein, pairwise data were obtained and plotted in Fig. 2. As observed, some chimeric β-galactosidases react poorly with all the mAbs (see M351VP1 and M962VP1), suggesting that in these cases, especially in M962VP1 protein, the antigenic site A could not be completely exposed on the protein surface. Like TSPA, some other chimeric β-galactosidases are well recognized by most of mAbs, with the exception of SB10 and SD6. In these cases (see M275VP1, M275LVP1 and to a lesser extent AB1 and AB1275VP1), there is a good correlation between IC_{50} values of TSPA and mutant β-galactosidases. Note that the coincidence is in fact promoted by both an efficient mimicry of the epitopes recognized by 3E5, 6D11, 7CA8, 7FC12 and 7JD11 and a poor reproduction of the epitope(s) recognized by SB10 and SD6. This is indeed the

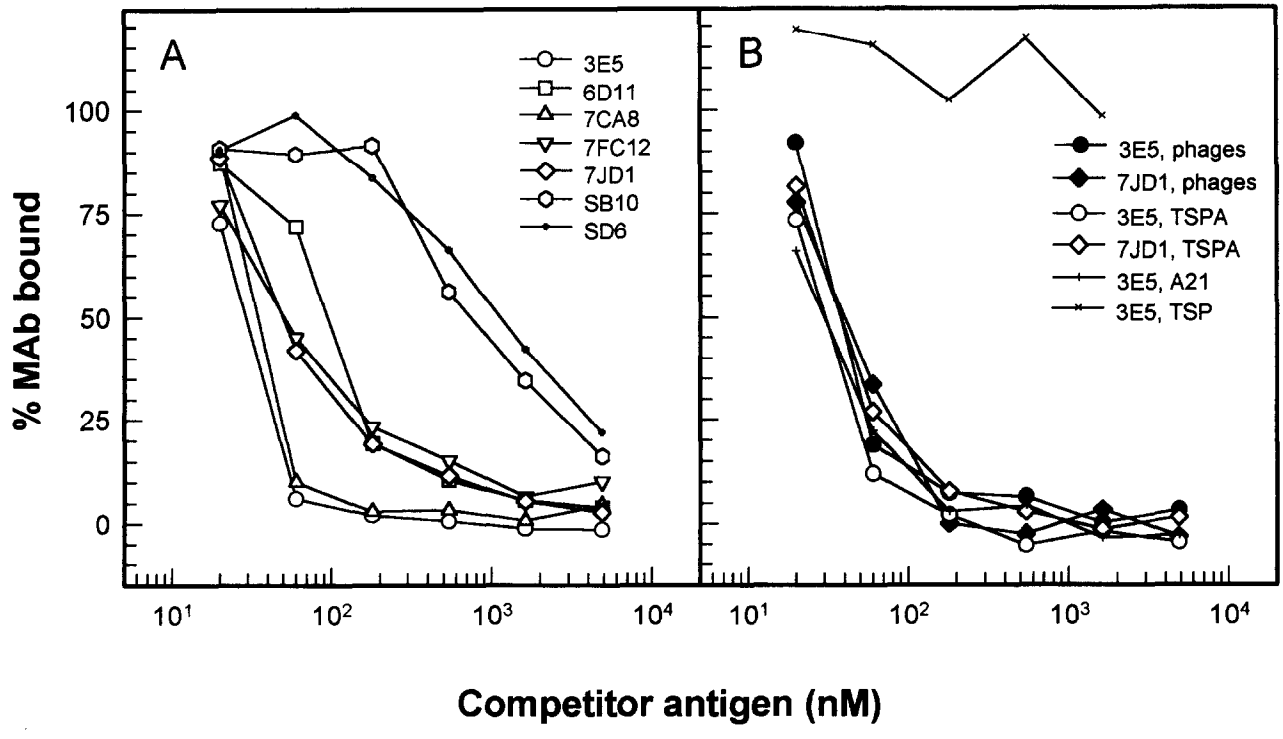

Fig. 1. A: Competitive ELISA with several mAbs directed to the FMDV site A, using 5 pmol of peptide A21 coupled to KLH as a bound antigen. The competitor antigen was soluble TSPA protein. B: Complete, infectious P22 chimeric particles containing TSPA (filled symbols) were used as competitors with mAbs 3E5 and 7JD1 and compared with TSPA protein (empty symbols). Free A21 and non-fused TSP were also included as a positive and negative controls, respectively.

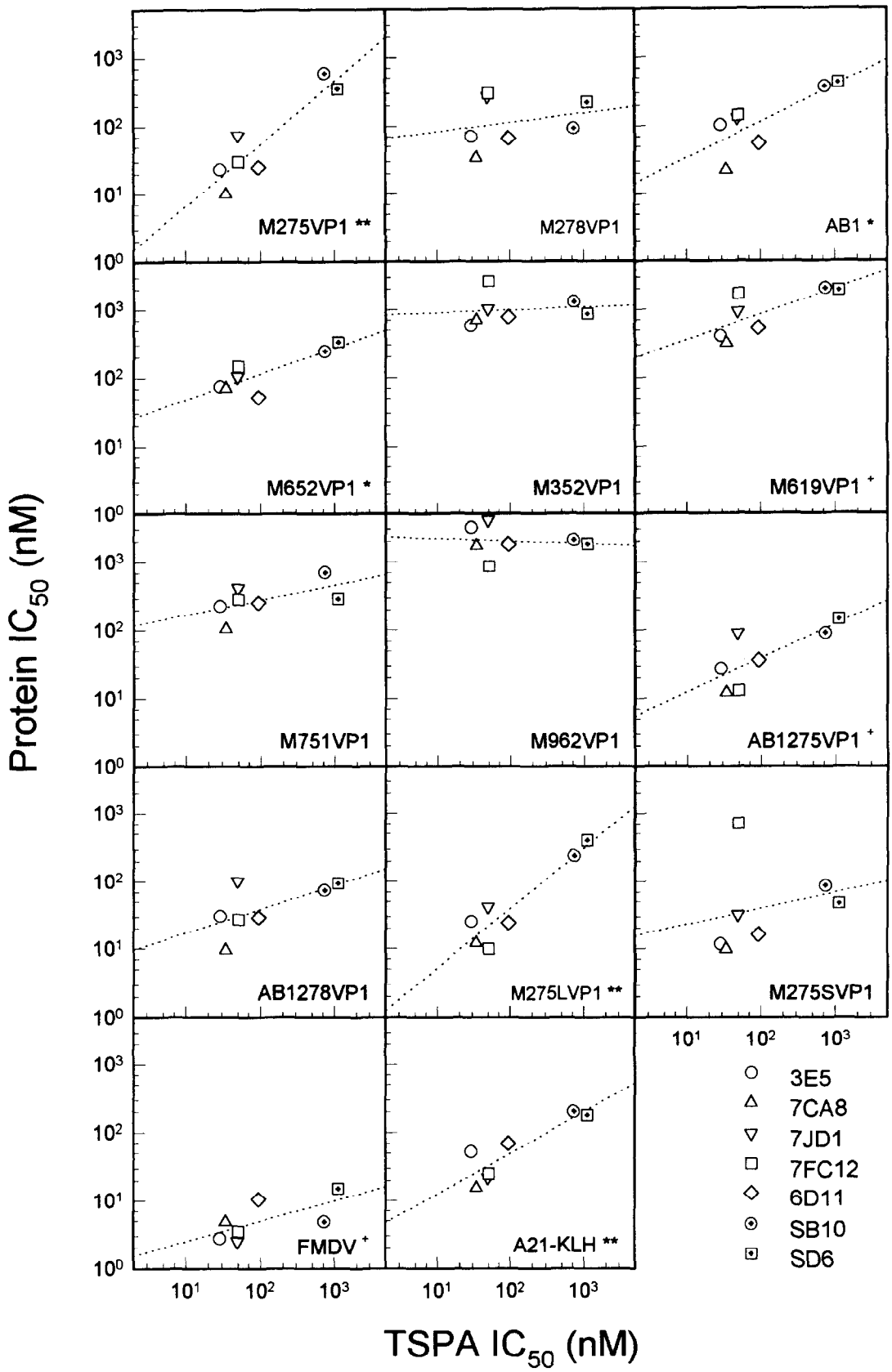

Fig. 2. A pairwise comparison between IC_{50} (given in nM) of TSPA and different chimeric β-galactosidases in competitive ELISAs, using A21-KLH as a bound antigen as explained in Fig. 1. Each point represents data obtained from one of the mAbs directed against site A. Regression lines for each panel are also shown. Symbols besides the names of proteins indicate the significance of the correlation coefficient: **, 99%; *, 95%; +, 90%; no symbol, <90%. Peptide A21 coupled to KLH and whole virus particles (FMDV) were also included as controls. The complete inhibition curves of chimeric β-galactosidases and control antigens will be given elsewhere (Benito et al., in preparation).

antigenic profile of the viral peptide in TSPA. As expected, the IC_{50}s obtained with the competing whole virus particles have the lowest values and do not discriminate between the two groups of B-cell epitopes, as TSPA and some β-galactosidases do. Other recombinant proteins, such as M278VP1 and M275SVP1, mimic the SD6–SB10 epitope better than TPSA.

In summary, the antigenic profile of the FMDV G-H loop, exposed on the virus surface, is mimicked by the viral peptide displayed on the C-terminus of P22 TSP. However, at least one B-cell epitope (SB10–SD6) is poorly reproduced and the reactivity of TSPA with both antibodies is low and similar to that of peptides presumably not solvent-exposed (i.e. M962VP1). On the other hand, the antigenic structure of TSPA is very similar to that observed in M275VP1. In TSPA, the viral peptide is fused to the C-terminus of TSP. This region is solvent-exposed and participates in the formation of a long β-helix turn [14]. In the C-terminus, the viral peptides in the interdigitated trimer are probably proximal to each other in the caudal fin of the TSP body [14], at the distal end of the P22 particle. In protein M275VP1, the viral peptide is inserted in a large, solvent exposed loop of the activating interface of β-galactosidase, which extends from one monomer to another and participates in the structure of the active site [20]. In this situation, the heterologous peptide seems to be very flexible and it might adopt a quasi-cyclic structure (Benito et al., unpublished results) that could be compatible with that observed in the reduced G-H loop on the virion surface [21]. Moreover, on the folded M275VP1 tetramer, the four copies of site A cannot be physically proximal. These results prove that it is possible to reproduce antigenic features of a viral peptide by display in completely different structural frameworks of recombinant proteins and that the symmetric disposition in a multimeric carrier is not necessarily critical for its antigenicity. The fact that the same epitopes are lost or maintained in TSPA and M275VP1 suggests that in the insertion sites of these two proteins, the viral segment adopts a functionally matching conformational spectrum.

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