Conclusions

1 A reliable SPR method for the kinetic analysis of binding between peptide antigens and immobilised antibodies has been established, despite the small size of the analytes. The interactions were well described by a simple 1:1 bimolecular interaction model and data were self-consistent, reproducible and in total agreement with previous competition ELISA screenings.

SPR kinetic analysis was, therefore, proven to be adequate for the functional characterisation of small FMDV peptide antigens.

2 Different combinations, reproduced by linear 15-residue peptides, of the four amino acid replacements found in the GH loop of FMDV isolate C-S30 were seen to be additive in ELISA and kinetic SPR assays. Whereas increasing the size of the C-S30 peptide did not cause any marked effect, overnight incubation with mAb in solution led to an antigenic reversion of peptide A15S30 towards mAbs 4C4 and 3E5, but not SD6. A similar effect was observed upon peptide cyclization. Solution NMR studies of both linear and cyclic C-S30 peptides showed that structural features formerly associated with peptide antigenicity were more pronounced in the cyclic peptide.

Although the FMDV GH loop is a continuous (i.e., linear) antigenic region usually well mimicked by linear peptides, conformation seems to have subtle, but important, effects in the reproduction of recognition events involving peptides derived from field isolate C-S30.

3 Antigenic FMDV peptides, comparable to or even better antigens than the wild type sequence, can be obtained by combination of adequate amino acid replacements. The peptide mutants display conformational and antibody – binding behaviour similar to those characterising the native peptide.

A stable mAb – peptide complex can be formed as long as key requisites are fulfilled, involving both residues committed in direct mAb – peptide contacts (^{141}Arg, ^{143}Asp, ^{146}His), and residues able to promote/stabilise a quasi-cyclic folding held up by a hydrophobic cavity (defined by positions 138, 144 and 147) and by intra-peptide hydrogen bonds delineating an open turn at the central region (positions 141, 142 and 143).