Solution Structure of a Retro-inverso Peptide Analogue Mimicking the Foot-and-Mouth Disease Virus Major Antigenic Site

STRUCTURAL BASIS FOR ITS ANTIGENIC CROSS-REACTIVITY WITH THE PARENT PEPTIDE*

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The major immunogenic site of foot-and-mouth disease virus (FMDV),† which is contained in the so-called G-H loop comprising amino acid residues 135–158 of capsid protein VP1 (viral capsid protein 1), is located in a disordered region on the surface of the particle (1). A single inoculum of a peptide corresponding to this region usually elicits levels of neutralizing antibodies that protect guinea pigs against a severe challenge with the cognate virus (2). In some instances, however, although the total reactivity of antibodies evaluated in immunochemical tests such as enzyme-linked immunosorbent assay is high, the level of neutralizing antibodies is low. This important problem has generated numerous studies aimed at enhancing the immunogenicity of this peptide with the hope of developing a peptide construct that could be used successfully as a synthetic vaccine (3).

Recently, we have shown that antisera raised in rabbits against peptides corresponding to the sequence 141–159 of two variants of serotype A cross-reacted strongly with the corresponding retro-inverso analogue peptides. Most importantly, the retro-inverso analogues, also called retro all-D or retro-enantiopeptides (4, 5), induced greater and longer-lasting antibody titers than did the respective parent peptides (6). Moreover, we showed with the FP variant analogue (which contains Phe and Pro residues at positions 148 and 153, respectively) that a single inoculation of the retro-inverso peptide elicited high levels of neutralizing antibodies that persisted longer than those induced against the corresponding L-peptide and protected guinea pigs challenged with the cognate virus (7).

The enhanced immunogenic activities of retro-inverso analogues may result from their higher resistance to proteolysis in biological fluids (7). However, this property does not explain why in immunochemical assays, retro-inverso peptides are often better recognized than the parent peptide by anti-virus, anti-protein, or anti-peptide antibodies with equilibrium affinity constants which can be increased by 10–100 in some instances (7–9).

To better understand the structural basis for antigenic mimicry between the L-peptide and its retro-inverso analogue, Carter et al. (10) recently analyzed the L- and retro-inverso peptides 141–159 of the SL variant (with Ser and Leu residues at positions 148 and 153) by NMR spectroscopy and restrained molecular modelling. The NMR structures of the peptides were determined in trifluoroethanol-d₂ (TFE-d₂) at different temperatures. As expected (because the retro-inverso and L-peptides share inherently chiral secondary structure elements) both peptides were found to wind in opposite directions. A detailed analysis of the results showed the presence of other different structural features between the peptides that exhibited only gross structural similarity. Thus, the L- and retro-inverso peptides maintain an approximately constant backbone conformation within the conserved RGD triplet (residues 145–147), which represents the principal site for virus attachment. However, the backbone of the RGD triplet is not equivalent in the L- and retro-inverso peptides. In the L-peptide, the RGD motif is enclosed in a type IV β-turn, whereas in the retro-inverso analogue it is part of a loose left-handed helix. Conformational variability is found about glycine 149 in both families of structures. Finally, a helical region encompasses residues 152–159 of the L-peptide and residues 150–159 of the retro-inverso analogue. In both cases, the helix is amphipathic but as indicated above, it is right-handed in the parent peptide and left-handed in the retro-inverso peptide analogue. Thus, a number of different structural features described for the L- and retro-inverso peptide 141–159 are particularly significant, and this...
observation does not support the fact that the retro-inverso peptide displays similar or superior cross-reactive antigenic activity compared with the L-peptide with regard to anti-virus or anti-protein antibodies. As suggested by Carver et al. (10), this discrepancy could be explained by assuming that the structures of the peptides in TFE do not correspond to those assumed by each peptide in aqueous solutions used to test their antigenic activity or in vivo when their immunogenic activity is investigated. In an attempt to rationalize our previous biological findings and explain why the retro-inverso analogue mimics the antigenic activity of the parent peptide, we decided to test whether anti-virus and anti-peptide antibodies effectively react with the peptides in TFE and determine the preferred conformers of the L- and retro-inverso peptides in aqueous solution.

MATERIALS AND METHODS

Peptides—Two natural (L-peptides) and two retro-inverso peptides corresponding to the VP1 region 141–159 of FMDV (serotype A, subtype 12, FP variant) were used in this study. The sequence of the parent peptide is \(\text{GSGQVDFGOSLFLRVARQ}^{159}\). This peptide contains the highly conserved RGD cell attachment site at residues 145–147. An additional cysteine residue was added at the N terminus of the peptides to allow their selective conjugation to the BIAcore sensor chips. CD and NMR studies were performed with peptides that contained no additional cysteine. The synthesis and purification of the peptides were described previously (6, 7). The N terminus of the L-peptides was acetylated, whereas their C terminus was left as -COOH. With respect to the native sequence, the N terminus (Gly \(^{141}\)) of the retro-inverso peptides was carboxamidated, and a 2-substituted malonic acid derivative was substituted for the native C-terminal L residue (7). Because the malonic acid derivative was introduced as a race mate during the synthesis, two diastereoisomers were generated. They were separated by HPLC, and only the most antigenically active diastereoisomer, which was slowly eluted (6), was considered valid. The purity of the L- and retro-inverso peptides was greater than 90% as checked by analytical HPLC. Peptide identity was verified by matrix-assisted laser desorption/ionization mass spectrometry using a protein time-of-flight apparatus (Bruker GMBH, Bremen, Germany) and gave the expected results for all peptides (data not shown). Control experiments were carried out with the L- and retro-inverso peptides of sequence IRGERA. These peptides, as well as the anti-IRGERA monoclonal antibody 4x11, were described previously (8, 9).

Antibodies—Anti-peptide antibodies used in this study were described elsewhere (6). They were raised either in guinea pigs immunized against the L- and retro-inverso peptides covalently conjugated to keyhole limpet hemocyanin in the presence of aluminum hydroxide as adjuvant or in rabbits immunized with peptides coupled to small unilamellar liposomes containing monophosphoryl lipid A as adjuvant (6).

Assay in TFE—Changes in the ability of peptides to bind antibodies in the presence of increasing concentrations of TFE were measured using the Pharmacia BIAcore biosensor instrument (Pharmacia Biosensor, AB, Uppsala, Sweden). The conventional immobilization technique was used to couple the L- and retro-inverso peptides to the sensor chips through their N-terminal thiol reactive groups (9). 267 and 185 pg of L- and retro-inverso peptides were respectively immobilized per mm \(^2\) (corresponding to 267 and 185 RU). After the peptide immobilization, the remaining reactive groups on the sensor surface were deactivated by a pulse with cysteine. The antibody preparation was then injected at a constant flow rate of 5 \(\mu\)l/min during 4 min at 25 °C, and the response expressed in RU was measured. After dissociating the bound antibody from the peptide surface in the presence of HCl (20 \(\mu\)l; flow rate, 5 \(\mu\)l/min) and washing, 20 \(\mu\)l of TFE in HEPES buffer (12.5%, v/v) were injected at a constant flow rate of 5 \(\mu\)l/min during 4 min at 25 °C. The peptide surface was then washed by injecting 10 \(\mu\)l of HEPES buffer (without TFE) at a constant flow rate of 5 \(\mu\)l/min for 2 min at 25 °C, and then the antibody preparation in HEPES (20 \(\mu\)l) was injected as described above, and the RU was measured. The same series of steps was repeated with TFE concentration (i.e., 25, 50, 75, and 100%). As a control, between each TFE step the peptide surface was regenerated (dissociation of the bound antibody) and washed, and the antibody was injected in HEPES without TFE. By measuring the RU between each step, we thus checked that the TFE treatment did not irreversibly alter the peptide surface reactivity.

CD Measurements—They were performed at room temperature on a Jasco J-710 spectropolarimeter flushed with nitrogen. Spectra were recorded in a wavelength interval of 250–190 nm using a 1-mm path length rectangular cell. Each spectrum was the average of five scans taken at a scan rate of 50 nm/s with a spectral bandwidth of 1 nm and corrected for base line using Jasco software. TFE titration was carried out by progressively dissolving the peptides in the appropriate solvent: (i) 0.1 mM sodium phosphate buffer, pH 5.7, and varying concentrations of TFE or (ii) 100% TFE. Typically, the peptide concentration was 0.2 mM. The results were expressed as mean residue ellipticity \(\theta\) having the units degrees cm\(^2\) dmol\(^{-1}\). According to the relation \(\theta = \theta_i/100lN\), where \(\theta_i\) is the measured ellipticity, \(l\) is the optical path length, \(N\) is the peptide concentration, and \(N\) is the number of amino acid residues in the sequence.

NMR Experiments—For the NMR experiments, 3 mM peptide was dissolved in 500 \(\mu\)l of phosphate buffer with 10% D\(_2\)O, pH 5.7 (noncorrected). All NMR experiments were recorded at 285 K on a 400 MHz DRX-Bruker spectrometer. The following homonuclear two-dimensional experiments were recorded: double-quantum-filtered \(\text{J}-\)correlated spectroscopy and total correlation spectroscopy with a mixing time \((\tau_m)\) of 70 ms and NOE spectroscopy 32 with mixing times from 100 to 400 ms. Water suppression was achieved using the WATERGATE sequence (11). Spectra were processed on Silicon Graphics Indy workstations using the XWINNMR 1.2 software (Bruker GMBH). Distance restraints were derived from cross-peak intensities of the NOE spectroscopy spectra with \(\tau_m = 200\) ms.

Structure Calculation—Intensities of peaks were extracted from the NOE spectra using KEASY software (12), and the interproton distances were calculated taking the distance of 1.78 Å between the two Pro\(^{152}\) \(\delta\)H protons as a reference. Distance restraints were assigned as strong, medium, and weak and set at intervals of 1.8–2.5, 2.5–3.5, and 3.5–5.5 Å, respectively. 

RESULTS AND DISCUSSION

Antigenic Activity of the L-peptide and Its Retro-inverso Anologue in Increasing Concentrations of TFE—Changes in the ability of peptides (corresponding to the sequence of the FP variant) to bind antibodies were measured using a biosensor instrument based on surface plasmon resonance. The peptides were coupled to the sensor chips through their N-terminal thiol reactive groups. After immobilization of peptide and deactivation of any remaining reactive groups on the sensor surface by a pulse with cysteine, the antibody preparation was injected at a constant flow rate of 5 \(\mu\)l/min during 4 min at 25 °C, and the response expressed in RU was measured. After dissociating the bound antibody from the peptide surface in the presence of HCl and washing, TFE in HEPES buffer (12.5%, v/v) was injected at a constant flow rate of 5 \(\mu\)l/min during 4 min at 25 °C. The peptide surface was then washed by injecting HEPES buffer (without TFE) at a constant flow rate of 5 \(\mu\)l/min during 2 min at 25 °C; the antibody preparation in HEPES (20 \(\mu\)l) was then injected as described above, and the RU was measured. The same series of steps was repeated for each TFE concentration (i.e., 25, 50, 75, and 100%). Control experiments performed to check that TFE treatment did not irreversibly alter the peptide surface reactivity are described under “Materials and Methods.” This protocol allowed us to study the effect of TFE on the antigenicity of the peptides without affecting the reactivity of antibodies that remained in the standard HEPES buffer, pH 7.4.
The data presented in Fig. 1 illustrate the reactivity of guinea pig and rabbit antibodies raised against the parent and retro-inverso peptides 141–159 with the L-peptide and its retroenantiomer as a function of TFE concentration. A slight decrease in antibody binding was observed when the peptides were in 12.5% TFE, and a sharp drop of binding was found when peptides were in 25–50% TFE. In general, guinea pig (Fig. 1, a and b) and rabbit (Fig. 1, c and d) antibodies did not react or reacted very weakly with peptides in 75 and 100% TFE. A control experiment with the hexapeptide of sequence IRGERA, known to remain unordered in 100% TFE (15), was performed in parallel. Using a monoclonal antibody induced against the L-hexapeptide (9) and the protocol described above, no change in antibody binding was found when the parent and retro-inverso IRGERA peptides were in HEPES or in HEPES containing 12.5–100% TFE (Fig. 1e). These results indicate that our procedure does not affect the reactivity of the antibody itself and that changes in antibody binding are only observed when the structure of the peptide becomes an α-helix in the presence of TFE. Interestingly, in another study (16), it was shown in enzyme-linked immunosorbent assay that when the native structure of peptides is an α-helix, certain monoclonal antibodies react better with the peptides dried from TFE onto the solid phase than with the same peptides adsorbed in aqueous solution.

Fig. 2 shows that when the parent and retro-inverso FMDV peptides were in 50% TFE, the time required to restore the structure recognized by antibodies was between 5 and 7 min in HEPES buffer at a constant flow rate of 5 µl/min. It is possible that when the dextran-linked parent and retro-inverso FMDV peptides are exposed to TFE, due to the local peptide density on the dextran matrix and the tendency of this FMDV peptide to form an amphipathic α-helix in TFE (10), helix bundle structures are formed, and a strong secondary structure stabilizing effect is exerted, explaining that several minutes (not milliseconds) are required to induce the folding to unfolding transition in aqueous solution. Such a stabilizing effect has been described by Mutter et al. (17) with template-assembled synthetic peptides involving helical peptides.

In summary, these results indicate very clearly that antibodies from immunized animals do not bind the L- and retro-inverso peptides 141–149 when they adopt an α-helical conformation. Furthermore the antibody reactivity was completely restored after a few minutes (>5) when the sensor chip-immobilized peptides were placed in a TFE-free buffer.Circular Dichroism Analysis—The conformational features of both the L- and retro-inverso peptides were investigated in solution by CD spectroscopy. An overlay of the CD spectra of the L- and retro-inverso peptides (0.2 mM) in 0.1 M phosphate buffer solution at pH 5.7 and in TFE is shown in Fig. 3a. As expected, both peptides exhibited almost symmetrical CD curves in phosphate buffer solution as well as in TFE.
aqueous solution, the spectra showed only a strong Cotton effect at 197 nm ($\theta = -1.08 \times 10^4$ and $1.04 \times 10^4$ for the L- and the retro-inverso peptides, respectively). Although such a CD signature is often assigned to a random-coil structure (18, 19), Siligardi et al. (20) argued for an equilibrium between a limited number of secondary structures that may include a left-handed extended helical conformation. In TFE, both the isomeric L- and retro-inverso peptides presented the typical pattern that can be assigned to the $\alpha$-helical conformation, with a trough at 222 nm and an extremum around 205 nm. However, in the case of the retro-inverso peptide, the intensity of the Cotton effect at 222 nm was stronger ($\theta = +6.20 \times 10^3$ versus $-4.49 \times 10^3$ calculated for the L-peptide). This result is in good agreement with previous NMR investigations performed in TFE-d$_2$ on a related antigenic peptide (variant SL), which revealed that the helical region present in the C-terminus at 222 nm (with respect to the parent sequence) of both peptides is more defined in the retro-inverso isomer (10, 21). To further characterize the structural behavior of the retro-inverso isomer in solution, an H$_2$O/TFE solvent titration was performed at room temperature. The presence of a plateau between 25 and 75% TFE, clearly deduced from the plot of $\theta$ at 222 nm versus TFE concentration (Fig. 3b), is indicative of a folding-unfolding pathway between three stable conformational components. It is worth noting that two plateau and four conformational states were observed by Siligardi et al. (20) in TFE titration of the related L-peptide encompassing residues 141–160 (variant SL) with a free N terminus. From our data, it appears that the retro-inverso isomer described in the present study shares similar conformational features with the L-peptides corresponding to variants SL and LP studied by Siligardi et al., which also involved a three-state process and a higher helical content in 100% TFE compared with the FP L-peptide (20, 22). However, during TFE titration of the retro-inverso peptide, Siligardi et al. observed two isodichroic points at 197 and 203 nm, which were not found in our case. The finding that some major conformational changes already occur when both the L- and retro-inverso peptides are in 5–25% TFE is in good agreement with the drop in antibody binding observed when the peptides were studied in this range of TFE concentration in mixed TFE buffer solutions (Fig. 1).

The results presented above strongly suggest that the folding pattern in the $\alpha$-helix observed in TFE for both the L- and retro-inverso peptides does not in fact correspond to the biologically relevant conformation(s) recognized by antibodies. It was thus decided to re-examine by NMR the structures of the L- and retro-inverso peptides in aqueous solution and in the absence of TFE.

**NMR and Molecular Modeling Analysis—**NMR data for both L- and retro-inverso peptides were collected on a DRX-400 spectrometer (Bruker) in aqueous solution (phosphate buffer, pH 5.7). A summary of NOE connectivities observed in the NMR spectra used for structure calculations of both peptides is shown in Fig. 4. The parent peptide 141–159 was found to be very flexible, and the NMR data then corresponded to a conformational averaging. However, the N and C termini exhibited different behavior that were analyzed using the program PROMOTIF (23). It was found that the N-terminal region (residues 141–149) was very flexible and contained no particular local structural motif. On the contrary, the C-terminal part (residues 150–159) appeared to be more rigid, with two distorted $\beta$-turns, although no permanent hydrogen bond was observed. The first $\beta$-turn involves residues 155–158 and is present in 81% of the structures obtained from the final run of DYANA calculations (Fig. 5). The second one involves residues 156–159 in 41% of the structures. Table I summarizes angle values and $C_{\psi}(i) - C_{\psi}(i+3)$ distances observed for these turns, and a superposition of the 10 structures with the best target functions is shown in Fig. 6a. The optimal fit of the backbone atoms corresponds to the sequence 150–158 and yields to a root mean square difference (r.m.s.d.) of 0.96 Å, whereas the 144–148 region (containing the RGD motif) gives an r.m.s.d. of 1.2 Å.

Pegna et al. (21) have reported the NMR structures of two VP1 peptides 141–160 (variants FP and SL) in TFE. Both peptides exhibited a helical motif from residues 151–158 and a loop encompassing the RGD motif (residues 145–147), whereas our results obtained in aqueous solution are rather in favor of two $\beta$-turns in the 155–159 region. Therefore, it appears that TFE is promoting a helical structure in the 141–159 (160) sequence. On the other hand it is notable that in good agreement with the results obtained in TFE by Pegna et al. (21), we could not find any particular element of secondary structure in the N-terminal part of the peptide (Figs. 5 and 6a).

The retro-inverso peptide 141–159 was subjected to the same NMR study in aqueous solution. Again, no permanent hydrogen bond was observed, but the analysis of the torsional angles and above all the $C_{\psi}(i) - C_{\psi}(i+3)$ distances revealed five atypical $\beta$-turns (Table II). As in the parent peptide, the C-terminal part of the retro-inverso peptide is folded by two $\beta$-turns involving residues 156–159 (56% occurrence; Fig. 5) and residues 155–158 (30% occurrence). Two other $\beta$-turns are also present in the central region of the peptide surrounding residues 150–151 and 152–153 with frequencies of 30 and 43%, respectively. Finally, the RGD motif is also involved in a $\beta$-turn present in 10% of the structures. Fig. 6b shows the optimal superposition of the backbone atoms for the 10 structures with the lowest target functions. The best fit corresponds to the sequence 150–158 and yields to an r.m.s.d. of 0.8 Å. We also measured an

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**Fig. 3.** Overlay of the CD spectra of the L- and retro-inverso peptides in 0.1 M phosphate buffer at pH 5.7 and in TFE. Mean residue ellipticity $\theta$ in degrees cm$^2$ dmol$^{-1}$ (a). Plot of $\theta$ (absolute values) at 222 nm versus the TFE concentration (b).
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r.m.s.d. of 0.9 Å on the positions of the backbone atoms for the sequence 144–148, containing the RGD motif. Carver et al. (10) found that in TFE, the C-terminal part of the retro-inverso peptide (variant SL) as well as the RGD motif were involved in helices. In contrast our results show that in aqueous solution, these two regions are involved in \( \beta \)-turns, illustrating again the helical promoter role of TFE. These conformational changes could explain why the parent and retro-inverso peptides are not recognized by antibodies when the peptides are in TFE.

**Final Comments**—Very few studies describing the structures of a peptide and its retro-enantiomer have been reported so far (10, 24, 25). It has been generally observed that the structures of L-peptides and their respective retro-inverso analogues are similar but not identical. The best topochemical similarity was achieved with a cyclo hairpin peptide and its retroenantiomer (24). Likewise, in the present study, only the C-terminal regions of the FMDV peptides, covering the residues 156–159, could be superposed (Fig. 7). Although the CO-NH bonds are in

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Fig. 4. Summary of nonambiguous observed connectivities for the parent (a) and retro-inverso peptide (b). For conventional reasons the numbering of residues was maintained in L- and retro-inverso peptides regardless the orientation of the peptide bonds. The strong, medium, or weak intensities of the NOE cross-peaks are indicated by the thickness of the lines.

Fig. 5. \( \beta \)-Turns found in the parent and retro-inverso peptides and frequency of occurrence. Only the parent peptide sequence is reported and used as a reference. The upper arrows (dotted) refer to the two \( \beta \)-turns characterized in the parent peptide, and the lower arrows (hatched) to the five \( \beta \)-turns found in the retro-inverso peptide.

**TABLE I**

| res. | 155 | 156 | 157 | 158 | 159 |
|------|-----|-----|-----|-----|-----|
| \( \Phi_2 \) | \(-141 \pm 1\) | \(-40 \pm 48\) | \(-86 \pm 34\) | \(-28 \pm 4\) | \(-83 \pm 27\) |
| \( \Psi_2 \) | \(-86 \pm 35\) | \(-40 \pm 48\) | \(-121 \pm 37\) | \(-5.3\) | \(-7.0\) |
| \( \Phi_3 \) | \(-86 \pm 35\) | \(-28 \pm 4\) | \(-5.3\) | \(-7.0\) | \(-5.3\) |
| \( \Psi_3 \) | \(-86 \pm 35\) | \(-28 \pm 4\) | \(-5.3\) | \(-7.0\) | \(-5.3\) |
| \( d(C_{\alpha}C_{\alpha+2}) \) | \(5.3\) | \(5.3\) | \(5.3\) | \(5.3\) | \(5.3\) |
| % | 81 | 41 | 41 | 41 | 41 |
opposite directions, the peptide backbones in this region assume closely similar conformations, and the side chains adopt the same orientation. This similarity may explain in part the cross-reaction of antibodies with these two peptides. Another important finding is that the retro-inverso analogue appears to be significantly more rigid than the parent peptide in aqueous solution, particularly in the region 145–154, which is unstructured and mobile in the parent peptide (Fig. 5). This feature supports our previous observation that the retro-inverso peptide 141–159 is better recognized than the parent peptide by anti-VP1 and anti-virus antibodies (6, 7). On the other hand, it has been reported that $\beta$-turns in antigenic peptides are the feature most commonly seen in peptide-antibody complex structures (26, 27). The three additional $\beta$-turns found in the retroenantio structure (Fig. 5) represent putative important nucleation sites that may be retained and further stabilized or rearranged into different structures when the peptide interacts with the antibody paratope. As far as the FMDV sequence studied in this work is concerned, our findings thus suggest that the retro-inverso strategy has proven to be useful not because it allowed the generation of a peptide whose surface closely mimics the L-peptide or the cognate region in the virus, but rather because it reduced the conformational space available to the peptide when it binds to the corresponding antibody.

**Table II**

| res. 1 | res. 2 | res. 3 | res. 4 | $\Phi_2$ | $\Psi_2$ | $\Phi_3$ | $\Psi_3$ | $d(C_\alpha C_\beta)$ | % |
|-------|-------|-------|-------|----------|----------|----------|----------|------------------|---|
| 156   | 157   | 158   | 159   | 112 $\pm$ 45 | $-7 \pm 62$ | 117 $\pm$ 38 | 70 $\pm$ 82 | 4.8–6.9 | 56 |
| 155   | 156   | 157   | 158   | 155 $\pm$ 40 | $-128 \pm 40$ | 112 $\pm$ 45 | $-7 \pm 62$ | 5.0–7.0 | 30 |
| 151   | 152   | 153   | 154   | 47 $\pm$ 14 | $-99 \pm 12$ | $-178 \pm 5$ | 6.6–7.0 | 43 |
| 149   | 150   | 151   | 152   | 134 $\pm$ 46 | $-113 \pm 19$ | 134 $\pm$ 18 | 46 $\pm$ 48 | 6.4–7.0 | 30 |
| 145   | 146   | 147   | 148   | 135 $\pm$ 48 | $-38 \pm 76$ | 163 $\pm$ 13 | $-59 \pm 44$ | 6.4–6.9 | 10 |

**Fig. 6.** Stereoviews showing ten superposed structures coming out from DYANA calculations with the best target functions. The L- and retro-inverso peptides present a very flexible N-terminal region (residues 141–150). The best fit was found for the 151–159 region with an r.m.s.d. of 0.9 Å for the parent peptide (a, blue) and 0.8 Å for the retro-inverso peptide (b, red). $C_\alpha$-$C_\beta$ bonds (thin lines) were added to display the side chain orientations. This figure was produced using the MOLMOL program (version 2.5.1) (14).

**Fig. 7.** Stereoview showing best fit superpositions of mean structure of parent (blue) and retro-inverso peptides (red). Because the peptide bonds were inverted, only the $C_\alpha$ were taken into consideration for superposition. Only the heavy atoms from residues 155 to 159 are shown. The mean structures were calculated using the MOLMOL program and minimized using the DYANA program (13).
In this respect, a detailed comparison of the L- and retroenantiomeric peptide structures in contact with the paratope of specific monoclonal antibodies will be of considerable interest.

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