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The HF-SCF energy of HIV-1 MNgp120 V3 hairpin loop conformers

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Abstract The purpose of this study is to analyze the structure of the V3 loop of the HIV-1 gp120 molecule at the atomic level. The total energy of each member of the antibody-complexed 16-mer V3 conformer data set of Sharon et al. (PDB 1NJ0) was determined by the Hartree–Fock-self-consistent field (HF-SCF) method and with the GROMOS96 force field. There was no correlation between the results of the classical GROMOS96 force field analysis and the ab initio HF-SCF quantum mechanical analysis of the energy of the V3-loop-peptide conformers. HF-SCF optimization (AM1) of conformer geometries yielded structures in which HIS315 is displaced from its original position in the combining site of human antibody fragment 447-52D, but with the hairpin turn intact. The hairpin shape of the V3 loop remained detectable, albeit distorted, even with perturbation by a lithium dicationic electrostatic force field and by substitution of the PRO320 at the crown of the V3 hairpin by a GLY. These data suggest that the hairpin conformation is at least partially stable to long-range electrostatic perturbations, either with or without PRO in the tip of the crown of the V3-hairpin loop.

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Introduction

Neutralizing antibodies directed against the V3 loop of gp120 block the attachment of HIV to the CCR5 and CXCR4 co-receptors required for virion penetration of target cells. [1, 2] The amino-acid sequence of the β-hairpin turn region of the V3 loop is relatively invariant among the HIV clades isolated from numerous patients. [3] For these reasons, the V3 loop of the gp120 protein is an important candidate antigen for the development of a protective, neutralizing anti-HIV vaccine.

The structure of the V3 loop in complex with human and with animal antibodies has been well characterized crystallographically [4, 5] and by NMR. [6] The available detailed structural studies provide parameters for a theoretical analysis of V3 as antigen. Theoretical analyses of V3 structure/function relations will increase understanding of the biology of this important region of the HIV virion and can facilitate the development of an anti-HIV vaccine. In this paper, we report a theoretical energy analysis of the 16-mer antibody-complexed PDB 1NJ0 [7] V3 loop peptide, at the atomic level.

Methods

All-atom, including hydrogen, atomic coordinates for 29 MN-strain HIV-1 gp120 V3-loop conformers (PDB 1NJ0) were obtained from the Protein Data Bank. [7] The net charge of each 1NJ0 V3-loop conformer, at 308 K and pH 5.5 [7] is +6, accounting for the protonated N-terminus, the deprotonated C-terminal carboxylate anion and the six protonated, cationic amino acid side-chain sites. According to the reported atomic coordinates, the six cationic amino acid residues were ARG311 LYS312 ARG313 HIS315 ARG322 and LYS328. The identical atomic coordinates were used for GROMOS96 force field and HF-SCF calculations.

The total energy of each V3 conformer was determined from the atomic coordinates with the GROMOS96 force field [8] implemented in vacuo by the Swiss-PdbViewer Version 3.7 with the 43B1 parameter set. [9] Restricted Hartree–Fock self-consistent

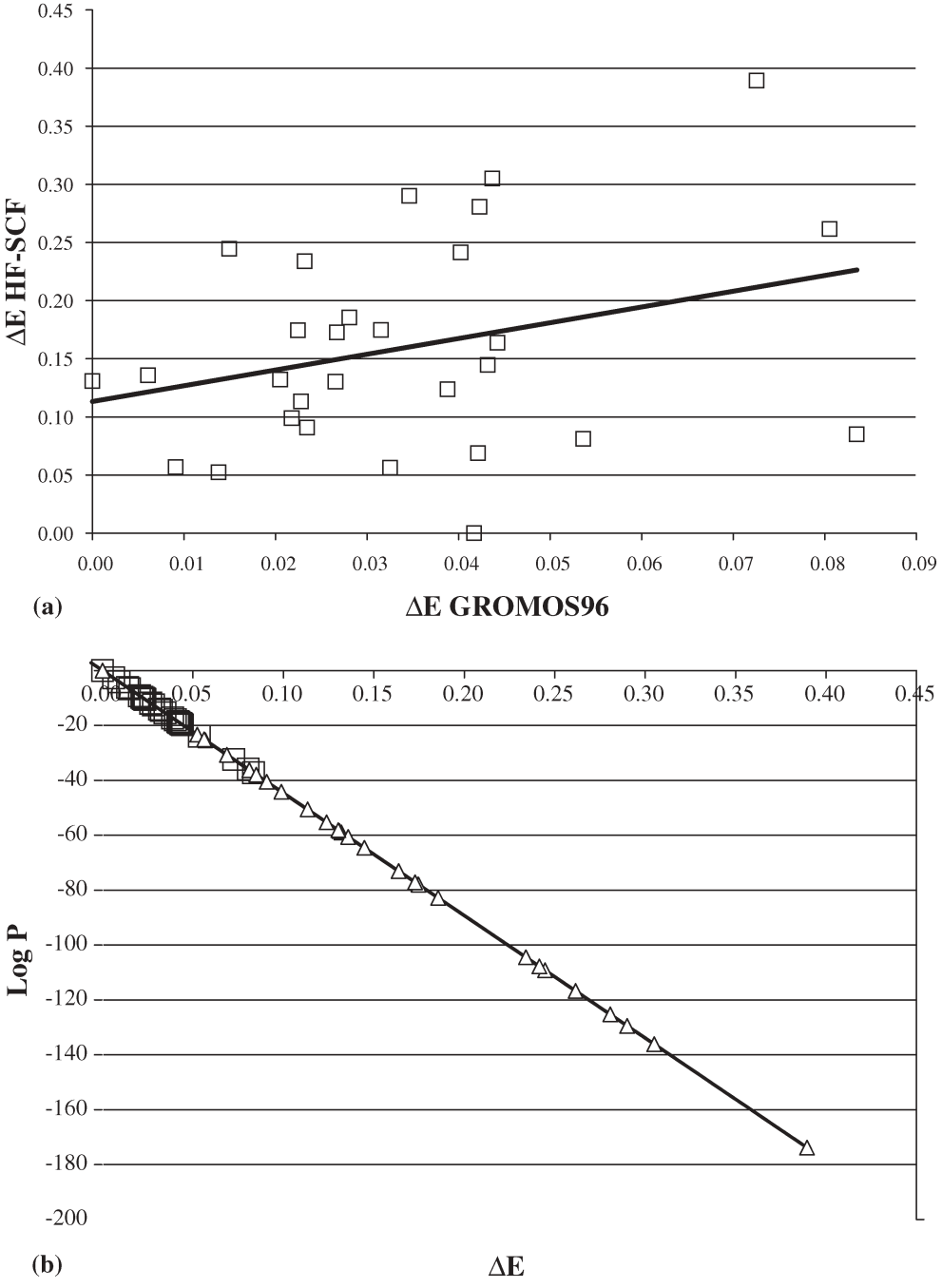

Fig. 1a, b Energy and relative Boltzmann probability of HIV-1 PDB 1NJ0 (7) gp120 V3 conformers. **a** GROMOS96 energy difference versus HF-SCF energy difference. The energy difference (ΔE) is the difference between the calculated energy (E) of each conformer and the lowest calculated energy (E_0) for each conformer data set. ΔE is in atomic units (a.u.). T=308 K. The linear correlation coefficient (r) between the HF-SCF ΔE energy differences and the GROMOS96 ΔE is not statistically significant (r=0.30, p>0.05, df=28). **b** Relative Boltzmann probabilities (P). The rectangles represent P values calculated from GROMS96 ΔE and the diamond shapes represent P values calculated from HF-SCF ΔE

field (HF-SCF) analyses were performed with the V3 atomic coordinates, in vacuo, using the US version of GAMESS [10] on a 12-node Xeon dual-chip Linux cluster with 4 GB memory in each node and a Myrinet interconnect. The HF-SCF energy of each of the 29 V3 conformers was determined with the MINI basis set. The HF-SCF energies of two of the conformers (Model 5 and Model 27) and of geometry-optimized Model 5 were also determined with the 3-21G** basis set.

Geometry optimizations were performed with a Pentium-4 optimized PC GAMESS version of GAMESS [11] using the semiempirical AM1 HF-SCF method, except for geometry optimizations in a lithium dication force field, for which the MNDO method was used.

The HF-SCF and GROMOS96 energy values were used to calculate Boltzmann probabilities [12] from $P=\exp[-(E-E_0)/kT]$, where E is the energy of each V3-peptide conformer, E_0 is the lowest (most negative) HF-SCF and GROMOS96 conformer energy obtained, k is Boltzmann's constant in atomic units (k=3.166793095×10^{-6} a.u./K) and T is the absolute temperature (308 K).

Ramachandran plots [13] were obtained with Molprobity [14] and the geometry of each conformer was evaluated with PROCHECK. [15, 16]

Differences (R) between atomic coordinates were calculated in Ångstroms, as $R=|r-r'|$. End-to-end distances, measured between the N-terminal atom and the C-terminal atom, and space-filling graphics were obtained with ViewMol3D. [17]

Table 1 Total HF-SCF energy of V3 conformers. Energy is in Hartee atomic units (a.u.)

Basis set	V3 conformer	Energy (a.u.)
MINI	Model 5	−6372.3183
MINI	Model 27	−6372.3643
MINI	Geometry optimized model 5	−6373.0607
MINI	Model 15	−6372.4493
MINI	Model 22	−6372.0598
3-21G**	Model 5	−6409.4157
3-21G**	Model 27	−6409.4452
3-21G**	Geometry optimized model 5	−6409.8732

The residue sequence numbering system used for V3 peptide 1NJ0 was: [7]ARG311 LYS312 ARG313 ILE314 HIS315 ILE316 GLY319 PRO320 GLY321 ARG322 ALA323 PHE324 TYR325 THR326 THR327 LYS328. This numbering system for the amino acid residues of V3 1NJ0 reflects the deletion of HIV-1 IIIB gp120 V3 residues GLN317 and ARG318 [18] in 1NJ0. For some of the geometry optimizations reported below, a glycine (GLY320) was substituted for the proline (PRO320) at the tip of the crown of the V3 hairpin loop. The stability of the hairpin was determined by geometry optimization in the presence of a force field generated by two lithium cations complexed to the terminal carboxylate anion of the V3 peptide.

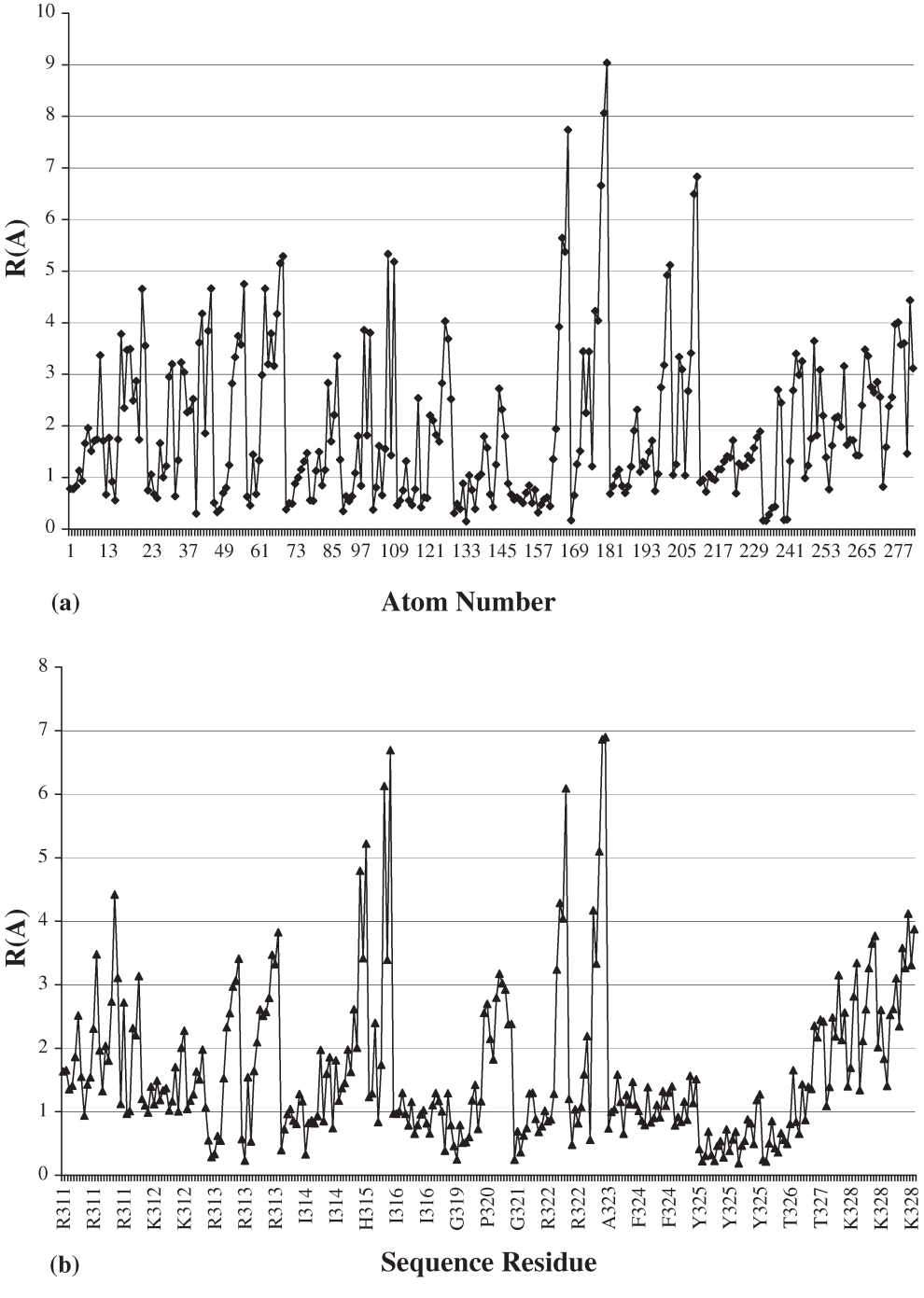

Fig. 2a, b Differences between atomic coordinates of V3 conformers. **a** Model 5 versus Model 27. **b** Model 5 versus geometry optimized Model 5. The magnitude of the difference (*R*) between each set of coordinates is in Ångströms. The abscissa of **a** shows the PDB atom number in the 1NJ0 peptide [7]. The abscissa in **b** shows the amino acid residues

Results

The conformer with the lowest HF-SCF energy was Model 15 and that with the highest HF-SCF energy was Model 22. In contrast, the conformer with the lowest GROMOS96 energy was Model 5 (E=–0.0896 a.u.) and that with the highest GROMOS96 energy was Model 27 (E=–0.0061 a.u.). Table 1 shows the HF-SCF energy for these V3 conformers and for the geometry-optimized Model 5 conformer. The end-to-end distances were 7.71, 6.40 and 4.62 Å for the Model 5 conformer, the Model 27 conformer and for the geometry-optimized Model 5 conformer, respectively.

Figure 1a shows that there was no significant correlation between the GROMOS96 energy differences ($\Delta E=E-E_0$) and the HF-SCF ΔE (r=0.30, p>0.05, df=28). The relative Boltzmann probability ratios (P) calculated from ΔE for the 29 PDB 1NJ0 V3 conformers are shown in Fig. 1b for GROMOS96 space and for HF-SCF space.

The differences between the atomic coordinates for Model 5 and Model 27, the two V3 conformers at the boundaries of the GROMOS96 energy data set, are shown in Fig. 2. Figure 2a is a comparison of the atomic coordinates of Model 5 with those of Model 27 and Fig. 2b is an atom-by-atom comparison of the coordinates of Model 5 with those of AM1 geometry-optimized Model 5.

Figure 3 shows Ramachandran plots for 1NJ0 Models 5, 7, 12, 22, 24, 25 and 26, i.e., for the seven V3 conformers for which some abnormality in the phi–psi angles was detected. The rank of the energy of these conformers in HF-SCF space was 13, 24, 2, 29, 18, 11 and 21 and 1, 5, 4, 27, 13, 18 and 14 in GROMOS96 space. PROCHECK analysis of these V3 conformers did not reveal any abnormal ω dihedral angles or any other systematic irregularities in molecular geometry.

Space-filling graphical representations and end-to-end distances for Model 5 (GROMOS96 lowest energy) V3 conformers and for a GLY320-substituted Model 5 conformer are shown in Fig. 4.

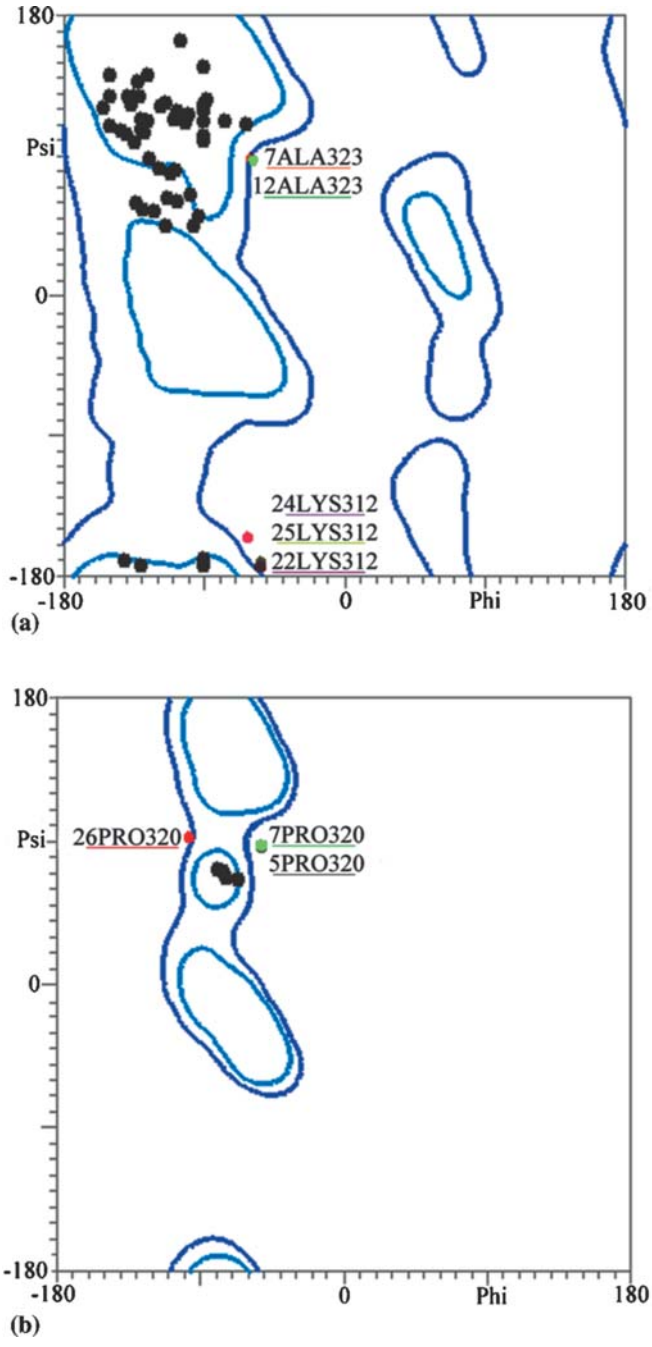

Fig. 3a, b Ramachandran plots of 1NJ0 gp120 V3 conformers. Plots are shown for the seven conformer models that registered abnormal plots. Conformer model numbers are shown before the abnormal amino acid sequence residue. Favored and allowed regions are indicated. **a** General except PRO. **b** PRO only

Discussion

There was neither qualitative nor quantitative agreement between the classical force field analysis and the quantum mechanical analysis of the energy of the PDB 1NJ0 HIV-1 MNgp120 V3 loop peptide conformers (Fig. 1a). The lack of concordance between the classical and the quantum mechanical approaches may be mitigated, in part, by solvent and temperature effects. It seems likely, however, that the disparate results between the classical and quantum mechanical energy calculations for V3 loop peptide represent the disparate assumptions of the two approaches.

The HF-SCF and GROMOS96 Boltzmann probabilities collapsed onto the lowest energy (E_0) and this collapse is orders of magnitude greater than can be accounted for by an increase in kT (Fig. 1b). Therefore, the atomic coordinates for each conformer represent a separate energy state, each in a local minimum on the potential energy surface. [19]

A smaller end-to-end distance in the V3-peptide conformer was correlated with a lower, more negative HF-SCF energy (paragraph 1 of the Results section and Table 1). The differences in atomic coordinates between the Model 5 and Model 27 V3 conformers are distributed throughout the V3 polypeptide chain but are predomi-

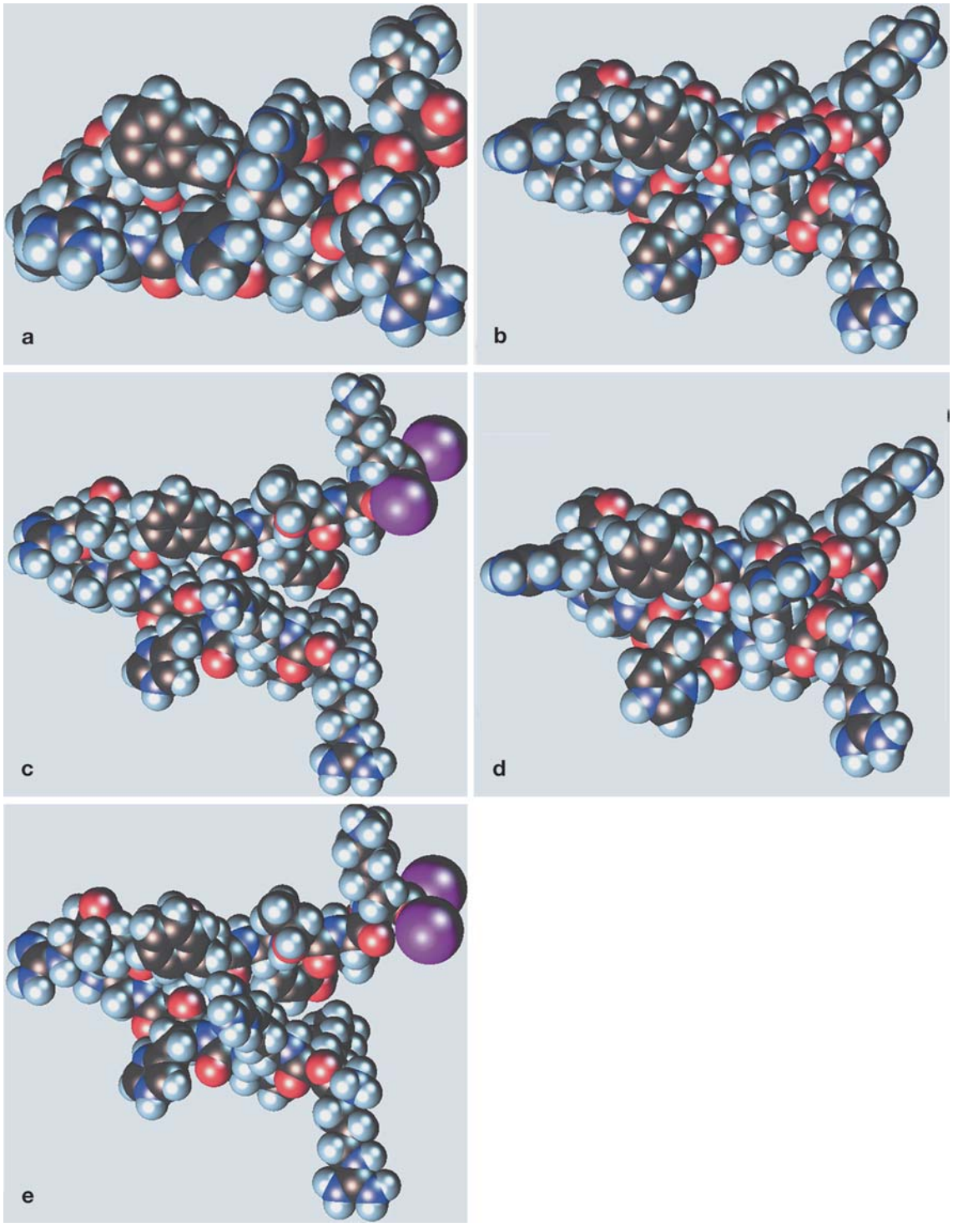

Fig. 4a–e Molecular geometry of HIV-1 V3 conformer Model 5 and a GLY320 substituted Model 5. Space-filling models were obtained with ViewMol3D [17]). *Red*=oxygen, *blue*=nitrogen, *black*=carbon, *white*=hydrogen and *purple*=lithium. End-to-end distance (*D*) was obtained with ViewMol3D and is in Ångströms. **a** Model 5: *D*=7.71 Å. **b** Geometry optimized Model 5: *D*=4.62 Å. **c** Geometry optimized Li2+Model 5: *D*=15.56 Å. **d** Geometry optimized GLY320 Model 5: *D*=4.74 Å. **e** Geometry optimized Li2+GLY320 Model 5: *D*=13.83 Å

nantly in the side chain atoms H167 and N180 at ARG322 (Fig. 2a). Relaxation of the conformation by geometry optimization of the V3 peptide was associated with further differences in coordinates at HIS315 side-chains. It should be noted that Sharon et al. [7] concluded from their NMR data that HIS315 was involved directly in the binding of human neutralizing antibody 447-52D to the V3 loop.

No significant deviations from peptide bond planarity were found in any of the V3 conformers (paragraph 4 of Results). However, Ramachandran plots of seven of the 29 V3 conformers showed at least one residue with abnormal geometry in the Ramachandran plot (Fig. 3) and one of the conformers (Model 7) evinced an abnormality in both PRO320 and in ALA323. The deviations from allowed φ–ψ angles were small, occurred in low energy and high energy conformers and did not correlate with either HF-SCF or GROMOS96 energy differences.

The atomic coordinates of the V3 loop peptide PDB 1NJ0 were obtained by Sharon et al. by solution NMR of the V3-peptide antigen bound to the single chain sc-Fv fragment of neutralizing antibody 447-52D. [6, 7] Lowering of the HF-SCF energy by geometry optimization (Table 1) showed that the V3 peptide is held by the antibody through the HIS315 side chain (Fig. 2b) in a high-energy position. The high-energy state of the antibody complexed V3-loop peptide can be achieved isothermally by entropy increases caused by solvent effects and by the conformational changes in anti-V3 antibody that accompany reaction with V3 epitopes. [20] The Ramachandran plot of geometry-optimized conformer Model 5 revealed no amino-acid residues outside the allowed regions (data not shown),

Optimization of the geometry of conformer Model 5 yielded a structure in which HIS315 is displaced (Fig. 2b) from its original position in the combining site of human-antibody fragment 447-52D, [6, 7] but with the characteristic type II β-hairpin turn intact (Fig. 4a and b). The hairpin shape of the V3 loop remained detectable, albeit distorted, even with perturbation by a lithium dicationic electrostatic force field (Fig. 4c and e) and by substitution of the PRO320 at the crown of the V3 hairpin by a GLY (Fig. 4d and 4e). The lithium cationic force field caused partial separation of the peptide segments of the hairpin, which was manifest in the base of the V3 loop, by a marked increase in the end-to-end-distance of the peptide (Fig. 4c and e).

The data presented in this paper suggest that the hairpin conformation, [6, 7] achieved by the HIV-1 V3 loop from its random conformation in solution, [21] is stable to long-range electrostatic perturbations, either with or without PRO in the tip of the crown of the hairpin. However, the fine tuning of the structural and functional effects of such perturbations may be modulated by amino-acid substitutions (e.g., TRP, GLN, SER, LEU, TYR, ALA, PHE, THR, VAL. MET, ARG) that have been shown to occur at the position of PRO320. [22]

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