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Discovery of Novel Promising Targets for Anti-AIDS Drug Developments by Computer Modeling: Application to the HIV-1 gp120 V3 Loop

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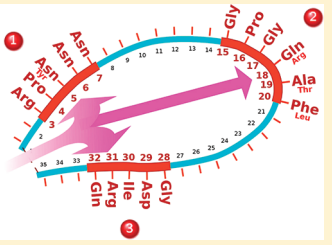
ABSTRACT: The V3 loop on gp120 from HIV-1 is a focus of many research groups involved in anti-AIDS drug studies, because this region of the protein determines the preference of the virus for T-lymphocytes or primary macrophages. Although the V3 loop governs cell tropism and, for this reason, exhibits one of the most attractive targets for anti-HIV-1 drug developments, its high sequence variability is a major complicating factor. Nevertheless, the data on the spatial arrangement of V3 obtained here for different HIV-1 subtypes by computer modeling clearly show that, despite a wide range of 3D folds, this functionally important site of gp120 forms at least three structurally invariant segments, which contain residues critical for cell tropism. It is evident that these conserved V3 segments represent potential HIV-1 vulnerable spots and, therefore, provide a blueprint for the design of novel, potent and broad antiviral agents able to stop the HIV's spread.

New promising targets for anti-HIV-1 drug developments



INTRODUCTION

In spite of disappointing progress for over more than a decade, studies on the design of anti-HIV-1 drugs and vaccines to protect against viral infection have still been focused on the gp120 third variable (V3) loop, which is a 35-residue long sequence that is frequently glycosylated and highly variable and contains a disulfide bond (e.g.,[1,2]). The V3 loop plays a central role in the biology of the HIV-1 envelope glycoprotein gp120 as a principal target for neutralizing antibodies for some HIV-1 isolates and as a major determinant in the switch from the nonsyncytium-inducing to the syncytium-inducing form of HIV-1 that is associated with accelerated disease progression.[1,2] HIV-1 cell entry is mediated by the sequential interactions of the envelope protein gp120 with the receptor CD4 and a coreceptor, usually CCR5 or CXCR4, depending on the individual virion. The V3 loop is critically involved in this process.[1,2]

Because of the exceptional role of the V3 loop in the viral neutralization and cell tropism,[2] knowledge of the 3D structure of the gp120 V3 loop is of particular importance. Currently, research teams engaged in anti-AIDS drug studies possess a definite set of experimental and designed data on the spatial structure of the HIV-1 V3 loop (e.g., ref 2); however, there are considerable contradictions between different structural models, which does not allow one to unambiguously answer the numerous questions concerning the modes of V3 functioning. It is clear that these structural discrepancies originate from the high sequence mutability of V3,[3] which provokes a wide range of its spatial folds (e.g., refs 4−6). Nevertheless, the occurrence of highly conserved residues within the V3 loop[3] allows one to suggest that they may preserve their conformational states in different HIV-1 strains and, therefore, should be promising targets for designing new anti-HIV-1 drugs. In this connection, the issue of whether these conserved amino acids may help to keep the local protein structure and form the structurally invariant segments of V3 exhibiting the HIV-1 vulnerable spots is very relevant. One of the plausible ways to answer this question consists of examining the V3 structures for their consensus sequences corresponding to the HIV-1 group M subtypes responsible for the AIDS pandemic followed by disclosing the patterns in the 3D arrangement of the variable V3 loops. Owing to the deficiency of experimental data on the V3 structures, these studies may be performed by homology modeling using the high-resolution X-ray and NMR-based V3 models as the templates.

In this work, the 3D structural models for the consensus amino-acid sequences of the V3 loop from the HIV-1 subtypes A, B, C, and D were built by bioinformatics tools to reveal common structural motifs in this functionally important portion of the gp120 envelope protein. To this effect, the most preferable 3D structures of V3 were computed by homology modeling and simulated annealing methods and compared with each other as well as with those determined previously by X-ray diffraction and NMR spectroscopy. Besides, the calculated V3 structures were also exposed to molecular dynamics computations, the findings of which were analyzed in conjunction with the data on the conserved structural elements of V3 that were obtained by collation of its static models.

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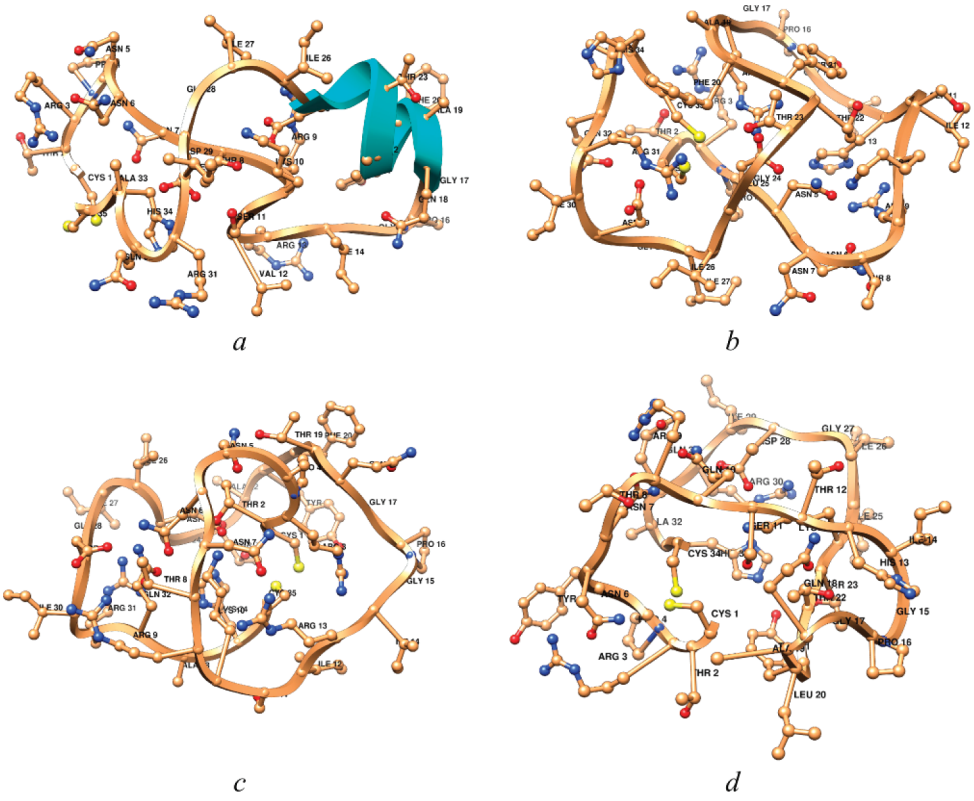

Figure 1. The most preferable 3D structures of the V3 loops from the HIV-1 subtypes A (a), B (b), C (c), and D (d) generated by homology modeling and simulated annealing.

As a matter of record, in spite of the high amino-acid sequence variability, the HIV-1 V3 loop was shown to exhibit less conformational mobility than previously appreciated and contain at least three structurally invariant portions, which greatly contribute to cell tropism. Consequently, these V3 sites provide a blueprint for the rational, structure-based design of novel, potent, and broad antiviral agents able to stop the HIV's spread.

METHODS

Modeling 3D V3 Structures. Modeling the 3D structures for V3 was implemented by several consecutive stages. In the first point, we made use of homology modeling in which the 3D structures of the V3 loop defined by NMR spectroscopy[4−7] and X-ray diffraction[8] were taken as the templates, and the consensus V3 sequences for the HIV-1 subtypes A, B, C, and D were used as the targets.[9] Homology modeling was performed by the MODELLER package.[10] The only restraint requiring the closing of the disulfide bridge between invariant residues Cys-1 and Cys-35[11] was imposed on the simulated models. At the same time, the sets including 100 models for each of the template were constructed. These structures were assessed by the DOPE statistical potential embedded in the MODELLER software. Then, the subsets containing the 10 best models were selected from each set for the energy optimization and final refinement by simulated annealing[12] in the GROMOS96 force field (parameter set 53A6)[13] using the GROMACS software tools for molecular design.[14]

To appreciate the quality of the computational models, the PROCHECK procedure was engaged in the studies.[15] In the closing stage, the best-energy V3 structures that satisfied the general criteria accepted in this program were selected for the final structural analysis and used as the starting points in the molecular dynamics simulations.

Identification of Secondary V3 Structures. To determine secondary V3 structures, the ϕ, ψ dihedrals were analyzed for the designed 3D structures by the criteria described in a study of Smith and co-authors.[16] The types of β- and γ-turns were identified within the classification of Hutchinson and Thornton.[17] To detect nonstandard β-turns, the additional information on the $C^{\alpha}_{i}...C^{\alpha}_{i+3}$ distances computed from the atomic coordinates of the simulated structures was employed.

Comparison of 3D V3 Structures. The values of root-mean-square deviations (rmsd) in atomic coordinates (cRMSD) were taken to evaluate the similarity of the structures in the Cartesian space (e.g., ref 18). To compare the structures in terms of the dihedrals, the rmsd between corresponding angles (aRMSD) were used as a measure of their conformational similarity in the angular space.[18] In each case, the values of rmsd were calculated both for the V3 structures and their individual fragments of different length, which comprised 4 to 9 residues.

Molecular Dynamics Computations. Molecular dynamics (MD) simulations were performed by the GROMACS computer package[14] using the GROMOS96 force field parameter set 53A6.[13] The starting 3D structures of the V3 loop were placed in a cubic box so that the smallest distance between its walls and the V3 atoms was greater than the half of the cutoff radius of the Coulomb and Lennard-Jones potentials fixed at 1.4 nm. Simple point charge water model[19] was utilized to set the parameters of explicit solvent on which the periodic boundary conditions were imposed in all directions. Before the MD computations, the initial V3 models were subjected to the procedure of energy minimization, which was realized in free space by 200 steps of the

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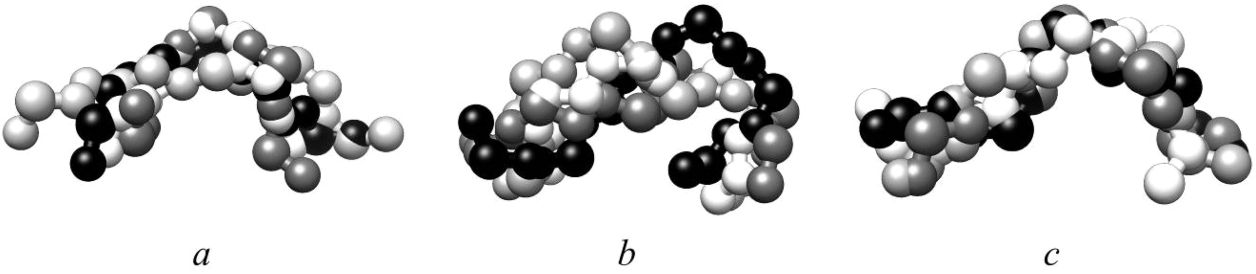

Figure 2. 3D structures for segments 3−7 (a), 15−20 (b), and 28−32 (c) of the V3 loops from the HIV-1 subtypes A, B, C, and D superposed with the best fitting. The averaged values of cRMSD, computed for all of the possible pairs in each set of the four simulated structures, amount 1.8 (a), 2.1 (b), and 1.4 (c) Å.

steepest descent method. The MD simulations were carried out at temperature 310 K during 10 ns time domain with 1 fs step at fixed pressure and number of atoms. To integrate the Newton's equations of motion, the common leapfrog algorithm was used. To control the temperature, the weak coupling scheme to an external bath[20] with 0.1 ps characteristic time was employed in the calculations. The system was linked to a "pressure bath" by exponential relaxation of pressure[20] with 1.0 ps time constant.

Every 10 ps, the geometric parameters of the MD structures and the data on their energy were recorded into the trajectory file. Comparison of the MD conformations between themselves and with the input structures was performed in terms of cRMSD and aRMSD.

The MD simulations were run in parallel on SKIF K-1000 computer cluster on 64 CPUs.[21]

RESULTS AND DISCUSSION

Figure 1 casts light on the 3D structures of the V3 loops from the HIV-1 subtypes A, B, C, and D generated by homology modeling and simulated annealing. All of the designed structures are located in the deep wells on the potential energy surface of V3. In addition, these structures meet the spatial restrictions imposed on the geometry of disulfide bridge Cys1-Cys35[11] and satisfy the general criteria commonly used for estimating the quality of local geometry of protein structures.[15] According to the data of the PROCHECK program,[15] the overpowering share of amino acids is localized in the analyzed structures within the energetically favorable regions of the Ramachandran plot, and their dihedral χ_1 angles are in line with the information appearing in the backbone-dependent rotamer library.[22]

Collation of the computed V3 structures indicates that they are dissimilar in the Cartesian space, which is supported by the values of cRMSD varying from 7.0 to 9.5 Å. The very same inference is readily apparent from comparison of the structures in the space of dihedral angles. In this case, the values of aRMSD lie in the range from 88 to 106°. These results are in harmony with those of the studies of Andrianov and co-authors[4−6] where, using comparative analysis of the NMR-based V3 structures for various viral isolates in different environments, this domain of gp120 was shown to be highly sensitive to changes of V3 amino-acid sequence involving a wide range of its spatial folds. However, confronting the individual V3 loop fragments of different length reveals that its segments 3−7, 15−20, and 28−32 exhibit close structural similarity in all of the designed structures (Figure 2). This finding is of great importance in connection with the data in the literature on the role of these V3 stretches in AIDS pathogenesis. In the list of the residues belonging to N-terminal site 3−7, it is necessary to note that Arg-3 is critical for binding to coreceptor CCR5 and heparan sulfate proteoglycans.[23,24] Pro-4 is also apparently important because it appears in all of the known V3 sequences of CCR5-tropic virions (e.g., ref 23). In addition, the structurally rigid stretch 3−7 contains one of the potential sites of the gp120 N-linked glycosylation[25] used by the virus for defense against neutralizing antibodies[26,27] and for maintaining its infectivity.[28−31] It is known (e.g., ref 29) that V3 asparagines in positions 6 and 7 encode a highly conserved glycosylation site for specific binding to the N-linked glycans, which modulates the interaction of the HIV-1 phenotypically diverse clades with CD4 and chemokine receptors and also serves to block access to the neutralization regions on gp120 of different isolates. As such, the data on the conformational mobility of V3 segment 3−7 provide evidence for the validity of anti-HIV-1 vaccine design strategy[32] aimed at the use of the N-linked glycosylation sites of the V3 loop. The relatively inflexible pentapeptide 15−20 region is a part of the V3 loop immunogenic crown,[33] which gives rise to overwhelming majority of the contacts with neutralizing antibodies and determines the specificity of their binding (e.g., ref 34). When analyzing this core V3 sequence, special attention should be paid to its tripeptide Gly-Pro-Gly, which is shared with practically all of the decoded primary structures of the HIV-1 principal neutralizing determinant.[3,35] The central proline of this conservative site presents the structurally inflexible amino acid of the V3 loop,[36−38] which affects the pathogenesis of the virus.[39,40] In particular, a single substitution for proline by alanine was shown to notably change the HIV-1 infectivity and immunogenicity.[39] Moreover, recent epitope mapping studies[41] revealed that most monoclonal antibodies seem to require the Pro residue, which forms the Gly-Pro-Gly-Arg β-hairpin turn in the V3 tip for binding. The structurally rigid pentapeptide 28−32 contains Asp-29, which contributes to cell tropism and cell fusion. Consistent with the data from a study of Hu and co-authors,[42] this Asp-29 residue influences the intensity of the CD4-activated gp120 protein binding to coreceptor CCR5.

It should be noted that the structural conservatism of V3 segments 3−7, 15−20, and 28−32 exists in the Cartesian space (Figure 2), but it is broken in the space of dihedrals. Comparative analysis of the dihedral angles of these segments in the designed V3 structures reveals significant differences between their local conformations: the values of aRMSD exceed 65° in all of the cases in question. This evidence signifies that the analyzed V3 sites are able to organize similar spatial folds by different combinations of their main chain torsional angles. Exactly the same conclusion concerning the V3 loop immunogenic crest was made in our

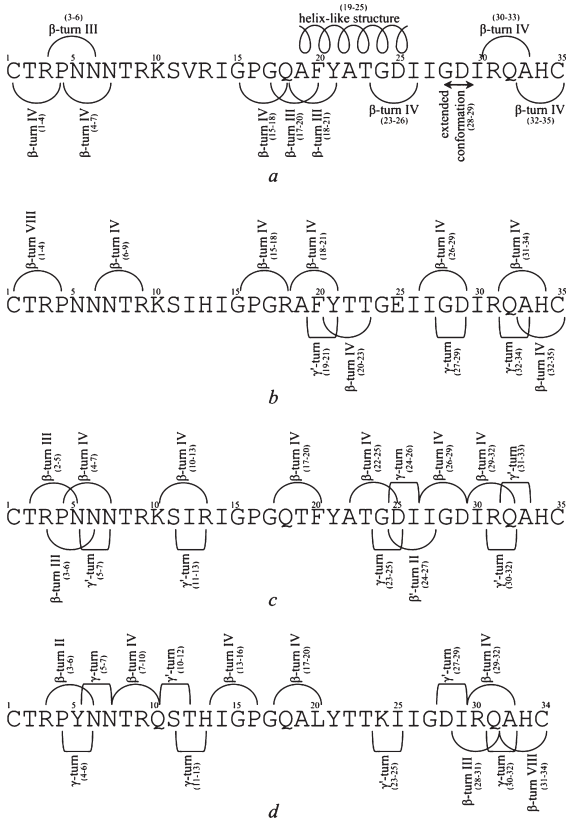

Figure 3. The elements of secondary structure identified in the best-energy conformations of the V3 loops from the HIV-1 subtypes A (a), B (b), C (c), and D (d).

previous study,[36] whereby V3 region 15−20 keeps the 3D structure in various HIV-1 isolates regardless of the conformational states of its residues. It is quite possible that the invariant 3D folds of V3 segments 3−7, 15−20, and 28−32 make the structural motifs, which are used by the virus to come in specific interactions with primary macrophages or T-lymphocytes; simultaneously, the high conformational mobility of their individual amino acids is likely to be required to provide energetically favorable contacts with coreceptors of these susceptible cells.

In the light of the findings obtained, it is of interest to compare the simulated V3 structures with those defined previously by NMR-based computer modeling[4−6] and X-ray diffraction.[8] This confrontation indicates that the compared structures exhibit the high structural variability both of the atomic coordinates and of the dihedral angles. However, as with above, V3 segments 3−7, 15−20, and 28−32 display the close similarity of the 3D backbone shapes and great discrepancies in the angular geometric space.

Examination of the calculated structures illustrates (Figure 3) that the V3 loop constitutes numerous β-turns, which are localized mainly in the central immunogenic region of V3 as well as in its N- and C-terminal segments. Analysis of Figure 3 makes it clear that the structural motifs appearing in the designed V3 structures are close to those identified earlier in the HIV-1 V3 loop by X-ray diffraction and NMR spectroscopy. With this analysis, the conserved residues of the N-terminal segment of V3 reside in β-turns of its polypeptide chain, which have been found in this portion of the V3 loops from the HIV-Haiti, HIV-MN, HIV-Thailand, and HIV-RF isolates.[4,6,7,43] Disclosure of the β-turn structures recurring in the N-terminal part of the V3 loop is of special interest because, as mentioned above, this V3 region gives rise to one of the potential sites of the gp120 N-linked glycosylation[25] and makes a contribution to cell tropism.[23,24] As follows from our simulations, the immunogenic apex of the V3 loops from the HIV-1 subtypes A and B forms a double β-turn structure (Figure 3). This observation matches the results of the NMR-based studies,[4,6] whereby V3 region 15−20 makes similar structures in the V3 loops from the HIV-Haiti and HIV-MN isolates. This is also in keeping with the data obtained in crystal for the V3 peptides in complexes with neutralizing antibodies[34,44,45] as well as for the HIV-1 JR-FL gp120 protein bound to CD4 and antibody X5.[8] At the same time, the local structure of V3 region 15−20 in the HIV-1 subtypes A and B conflicts with the conformation predicted for the virus clades C and D where this V3 site adopts a single β-turn 17−20 (Figure 3). These data pointing to the conformational variability of the V3 immunogenic tip go with those derived from the NMR and X-ray studies of the V3 peptides, which imitate this segment of the gp120 protein. In particular, the NMR studies display in this stretch type II β-turns,[46,47] both I and II β-turns,[48−51] nonspecific β-turn,[52] and a double β-turn[53] close to the structure revealed in crystal for the V3 peptides bound to different neutralizing antibodies.[34,44,45] Simultaneously, the X-ray studies indicate that the V3 immunogenic apex can adopt at least two different conformations, one of which yields a double β-turn II−III,[34,44,45] whereas the other exhibits a single β-turn in the invariant Gly-Pro-Gly-Arg site.[54]

Thus, the joint analysis of the calculated and experimental data proves again that the V3 loop immunogenic crest forms similar 3D backbone shapes in various HIV-1 isolates. However, this cryptic site of gp120 manifests the high conformational mobility of its individual amino acids, which results in different secondary structures. Structural rearrangements of this core V3 sequence are most likely to be used by the virus to escape the effect of neutralizing antibodies and other external agents.

According to the calculated data, C-terminal region of the HIV-1 V3 loop is in favor of forming the coiled conformations (Figure 3). This observation agrees with the NMR-based theoretical findings[7,43,55] as well as with the NMR and CD spectroscopy data[48] indicating that C-terminal V3 region longs for a helix-like structure. In addition, the 3D structure of the consensus CCR5-tropic V3 loop, which was studied by NMR spectroscopy in water and in a water/trifluoroethanol mixed solvent,[56] suggests a partial formation of a helix in the C-terminal region that is entirely formed on addition of trifluoroethanol. The amphipathic C-terminal helix, also appearing in other examined isolates,[7,55] was supposed to be an important feature for the functioning of the V3 loop.[56]

Analysis of the designed structures also shows that, in addition to β-turns, the HIV-1 V3 loop may expose such structural motifs as γ-bends (Figure 3) rarely appearing in proteins. These structural elements, which may be also important for V3 functioning, have been previously found in the NMR-based V3 structures of the HIV-Thailand and HIV-RF isolates[7] as well as of a synthetic peptide rp70 imitating the HIV-1 subtype B V3 loop.[43]

The data on the secondary V3 structure given above support the idea that the β-turns, recurring in the V3 loop regardless of the HIV-1 faces and environmental changes, may serve as the structural framework providing the HIV-1 coreceptor usage. Therefore, these conserved structural elements contributing to cell tropism represent potential HIV-1 weak points, which exhibit promising new targets for anti-AIDS drug development.

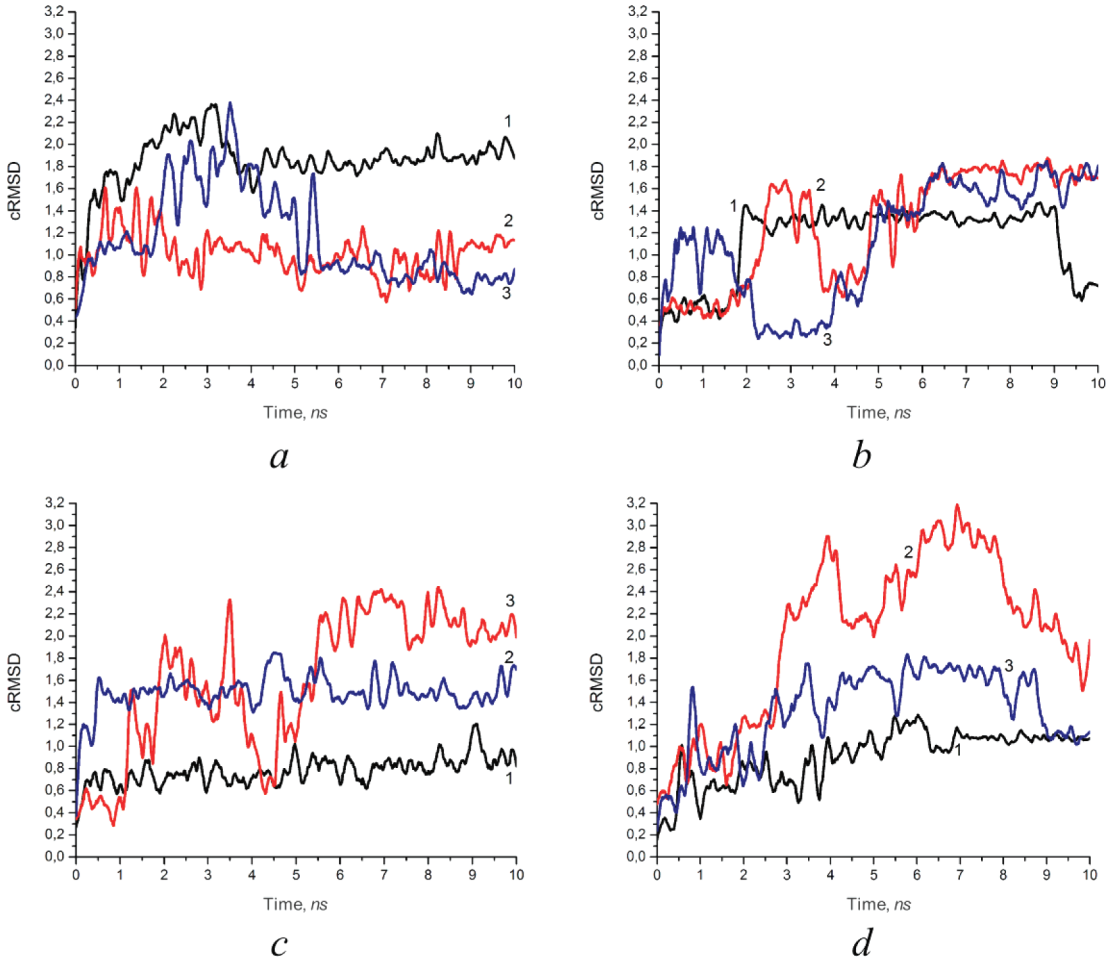

Figure 4. The time dependence of the cRMSD values (Å) computed between all of the MD conformations and the input structures of the V3 loops from the HIV-1 subtypes A (a), B (b), C (c), and D (d) for segments 3−7, 15−20, and 28−32 (graphics 1, 2, and 3, respectively). For better representation, each series is smoothed over 25 neighboring points. The averages of the cRMSD calculated for all the structures of the MD trajectories come to the following values: for segment 3−7 − 1.9 Å (a), 1.1 Å (b), 0.8 Å (c), and 0.9 Å (d); for segment 15−20 − 1.0 Å (a), 1.3 Å (b), 1.6 Å (c), and 2.0 Å (d); for segment 28−32 − 1.2 Å (a), 1.1 Å (b), 1.5 Å (c), and 1.3 Å (d).

An insight into the data of MD simulations enables one to make a conclusion analogous to that resulting from collation of the designed and experimental V3 structures. Inspection of the MD trajectories for the V3 loops from the HIV-1 subtypes A, B, C, and D testify to their high structural flexibility. The peak values of cRMSD between the dynamic and starting structures vary as 4.5...10.4 Å, and the corresponding values of aRMSD fall in the range of 78...94°. Investigation of the dynamic behavior for the V3 loop segments of different length displays that, for the most part, they are highly mobile. Nevertheless, analysis of the MD data makes it possible to reveal a series of exceptions to this observation. As would be expected, these exceptions concern V3 segments 3−7, 15−20, and 28−32, which have been identified here to be the structurally invariant sites of the V3 loop. Analysis of Figure 4 indicates that these V3 regions do not go through substantial structural rearrangements during the MD simulations. When examining the time conformational changes of V3 segments 3−7, 15−20, and 28−32, it is important to note that, in spite of holding the 3D shapes, their individual residues show the high amplitudes of fluctuations of the dihedral angles. For the V3 segments of interest, the peak value of cRMSD averaged over all of the structures of the MD trajectories aggregates 2.0 Å, whereas the corresponding minimal value of aRMSD comes to 66°. These data fall into line with the conclusion that has been made above from comparative analysis of the static V3 structures: the functionally meaningful V3 segments 3−7, 15−20, and 28−32 retain the 3D main chain folds despite the high conformational mobility of their single amino acids.

Probably, the time domain used in the above MD simulations may be insufficient to entirely scan the conformational space of V3. However, the findings of these calculations, which were obtained based on the four highly diverse starting points presenting the static models of the V3 loops (see the Methods section), clearly testify to their similar dynamic characteristics in all of the cases of interest. In addition, these observations are typical both for the intact V3 loop and its individual segments of different length, allowing one to believe that our MD simulations adequately describe the general dynamic features of this site of gp120.

The data obtained amplify the findings of a recent study of Almond and co-authors[57] in which the most variable sequence positions in the crown of the V3 loop were shown to cluster to a small zone on the surface of one face of the V3 loop hairpin conformation. These findings present a novel visualization of the gp120 V3 loop, specifically demonstrating a superiority of

conserved 3D structure in this highly sequence-variable region. In addition, four conserved structural elements within the crown of the V3 loop were also found by a systematic structural analysis of representative human anti-V3 monoclonal antibodies in complex with V3 peptides.[58] These V3 regions which are targeted by cross-clade neutralizing human antibodies are supposed to provide a pattern for the development of vaccine immunogens that could elicit broadly cross-reactive protective antibodies.[58] Moreover, some of these conserved V3 sites do not involve the Gly-Pro-Gly-Arg/Gln immunogenic tip and are common for non-B subtypes. These results taken together with the data of the present study support a structural rationale for the development of novel anti-AIDS drug candidates that target the invariant elements of the HIV-1 V3 loop.

CONCLUSIONS

In this way, the data on the structure of the HIV-1 V3 loop clearly point to the presence of conserved structural motifs within V3, which explains its role in binding to the virus coreceptors. In particular, the findings obtained in the present study prove convincingly that the V3 domain forms at least three relatively rigid portions, which include residues crucial for cell tropism. Furthermore, with this study, the biologically meaningful residues of the identified conserved stretches reside in β-turns of the V3 polypeptide chain, which may be used by the virus as docking sites for specific and efficacious intermolecular interactions. Therefore, these conserved V3 regions represent potential HIV-1 weak points most suitable for therapeutic intervention. In this context, the strategy for anti-HIV-1 drug discovery aimed at the identification of coreceptor antagonists that are able to efficiently mask the structural motifs of the V3 loop, which are conserved in different viral strains, is highly challenging. To solve this problem, an integrated computational approach involving theoretical procedures, such as homology modeling,[10] molecular docking (e.g., ref 59), molecular dynamics (e.g., ref 14), QSAR modeling (e.g., ref 60), and free energy calculations (e.g., ref 61), should be of great assistance in the design of novel, potent, and broad antiviral agents.

Finally, the above 3D structural models also provide a productive basis to gain a better insight into the principles of functioning of the V3 loop. These models, therefore, may be applied in future studies to investigating the structure−function relationships of V3 as well as to examining the effects of mutations on its conformational features and physicochemical properties, which is required for successful achievement of anti-AIDS drug design projects focused on the HIV-1 gp120 protein.

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