ISSN 0006-2979, Biochemistry (Moscow), 2006, Vol. 71, No. 8, pp. 906-914. © Pleiades Publishing, Inc., 2006.
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Determination of Structurally Conservative Amino Acids of the HIV-1 Protein gp120 V3 Loop as Promising Targets for Drug Design by Protein Engineering Approaches

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Received January 31, 2006
Revision received March 20, 2006

Abstract—Based on the published NMR spectroscopy data, three-dimensional structures of the HIV-1 gp120 protein V3 loop were obtained by computer modeling in the viral strains HIV-Haiti and HIV-MN. In both cases, the secondary structure elements and conformations of irregular stretches were determined for the fragment representing the principal antigenic determinant of the virus, as well as determinants of the cellular tropism and syncytium formation. Notwithstanding the high variability of the amino acid sequence of gp120 protein, more than 50% of the V3 loop residues retained their conformations in the different HIV-1 virions. The combined analysis of the findings and the literature data on the biological activity of the individual residues of the HIV-1 V3 loop resulted in identification of its structurally conservative amino acids, which seem to be promising targets for antiviral drug design by protein engineering approaches.

DOI: 10.1134/S000629790608013X

Key words: human immunodeficiency virus type 1, protein gp120, V3 loop, spatial structure, computer modeling, drugs

Study of the spatial structure of the HIV-1 envelope proteins is an extremely urgent problem, and its solution can be fundamental for antiviral drug design. This problem is especially difficult because of the high variability of the HIV-1 envelope proteins. Therefore, many researchers are now engaged in determination of the conservative regions of the virus and their role in the virus functioning. In particular, the principal neutralizing determinant (PND) of HIV-1 (the gp120 protein V3 loop) is shown to produce a hypervariable variant [1], but, nevertheless, it tends to preserve individual residues on the N- and C-ends and also in the central region which forms the HIV-1 immunodominant epitope (IDE) [2]. Due to immunochemical features, the V3 loop may be considered as a promising fragment for designing drugs for prevention and treatment of AIDS [3]. Thus, antibodies neutralizing the viral activity are shown to interact only with the conservative stretch of this loop [4, 5]. The binding of antibodies inhibits the virus penetration into the cell, but fails to prevent its interaction with the primary receptor CD4 [6-8]. In addition to the IDE, the protein gp120 region under study also produces the main determinants responsible for cell tropism and the syncytium formation [9-13]. The presence of conservative amino acids, which are constituents of the biologically active stretches of the fragment, suggests that they can be important contributors to specific functional features of the virus manifesting themselves during the different stages of its replicative cycle.

Because of the important role of the V3 loop in HIV-1 functioning, it is attracting intent attention of researchers engaged in drug design by protein engineering approaches [3]. Obviously, such works require data on the fine structure of the V3 loop.

Initially, the three-dimensional structure of the V3 loop was not established by X-ray crystallographic analysis of gp120 protein [14], but later it was interpreted with low (3.5 Å) resolution [15]. However, some structural models are known [16-20] which more accurately

Abbreviations: HIV-1) human immunodeficiency virus type 1; PND) principal neutralizing determinant; IDE) immunodominant epitope; TFE) trifluoroethanol.

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describe conformations of V3 loop synthetic fragments in a crystal complexed with neutralizing antibodies. According to these models, the HIV-1 IDE (the hexapeptide G-P-G-R-A-F) can have, at least, two conformations, one of which forms a double β-turn II-III (antibodies 50.1 [16], 59.1 [17], and 83.1 [19]), and the other forms a single β-turn I (antibody 58.2 [18]). X-Ray studies of chimeric rhinovirus MN-III-2 [21], which includes the HIV-1 main immunogenic stretch, have shown that, on incorporation into a foreign peptide chain, it acquires the double β-turn I-I conformation resembling the structure of the complex of the V3 loop peptides with antibody 58.2 [18]. By NMR spectroscopy, the V3 loop was shown [22-29] to take in aqueous solution an unordered conformation with a clearly pronounced population of reverse turns on the invariant tetrapeptide G-P-G-R. But the quantitative identification of these turns gave ambiguous results. Thus, according to works [22, 23], the conformational parameters of the central dipeptide P-G fitted the β-turn II structure. However, further studies revealed that on the V3 loop stretch under consideration other states could also be realized: the dynamic mixture of two conformers, such as β-turns I and II [24-27], an atypical β-turn [28], and also double β-turn [29], similar to that detected by X-ray crystallographic analysis in the peptides complexed with the Fab-fragments of the monoclonal antibodies (50.1 [16], 59.1 [17], and 83.1 [19]).

Thus, the literature presents insufficient and sometimes contradictory information about the spatial structure of the HIV-1 PND. Moreover, in the majority of the above-cited works, the structure of the HIV-MN V3 loop was analyzed of the virus strain isolated from a Minnesota (USA) resident. Certainly, to have information for drug design based on data on the spatial structure and conformation features of the HIV-1 V3 loop, it is extremely pressing to study the structural features of this loop in the virions from various areas of the world.

The present work continues our earlier theoretical studies [30-38] on the spatial structure of the HIV-1 V3 loop based on the NMR spectroscopy data. The purpose of this work was to determine the three-dimensional structures of the gp120 protein V3 loop in the HIV-Haiti and HIV-MN virions and compare their conformational parameters in order to detect conservative elements of the structure and individual amino acids retaining the conformation in the two modifications of the virus.

To attain this aim using the NMR spectroscopy data [22, 39], the spatial structures of the V3 loop in the HIV-Haiti and HIV-MN strains were computed in the mixture of water–trifluoroethanol (TFE) (7 : 3). This mixture is a solvent that imitates physicochemical properties of water surrounding the fragment when it is in a direct contact with the cell membrane. In both cases elements of the secondary structure and conformation of irregular stretches were established. The structural parameters of the HIV-Haiti and HIV-MN V3 loop were also compared in the geometric spaces of the internal rotation angles and Cartesian coordinates of the atoms, and this allowed us to identify the structurally stable and labile amino acids of the V3 loop. The structure–function dependence on the HIV-1 region producing the viral PND and the determinants responsible for the cell tropism and syncytium formation were also studied.

The advantage of this scheme of the study providing for the determination of structurally conservative amino acids of the HIV-1 PND has been supported by conclusions of some experimental works [12, 17, 40-42], which have shown that individual point amino acid substitutions in the V3 loop limits can markedly affect the infectivity of the virus, its immunogenicity, and the cross-reactivity of neutralizing antibodies.

MATERIALS AND METHODS

Modeling of three-dimensional structures. The modeling of three-dimensional structures of the V3 loop in the HIV-Haiti and HIV-MN virions included four successive stages: calculation on the basis of NMR spectroscopy data [22, 39] of weighted average values of dihedral angles ϕ and ψ of the amino acid residues in the fragment; generation of the spatial packing of the fragment main chain with closing of the disulfide bridge between the N- and C-terminal residues of cysteine; side chain reconstruction; energy optimization of structural parameters of the molecular models.

In the first stage, the approach described in [43-45] was used which included a probability model of the protein conformation and, based on the NMR spectroscopy data, the weighted average values of the molecule dihedral angles were calculated directly (without constructing a three-dimensional structure). These values were statistically analyzed having in mind the empirical function of the internal rotation angle distribution. For the calculations, the CONFNMR-2 computer program was used [31]. Based on the spectral information about *d*-connectivities of every amino acid residue [46], the region of its spatial location (ϕ, ψ) was determined, and then, taking into account the additional experimental information, the most probable values of the dihedral angles and standard deviations were calculated [43].

The conformations established on the basis of NMR data were considered as starting ones at the second stage of the calculations which resulted in the spatial structures of the main chain of the HIV-Haiti and HIV-MN V3 loop. At this stage, structures were generated which fitted the prescribed local minima of the individual amino acid residues and geometry of closing the S–S-bridge C-1–C-35 [47]. In these calculations, a modified version of the SE computer program [45] was used, which combined the energy minimization approaches adapted to the ECEPP2/3 force field [48] with the Monte Carlo proce-

dure [49]. To provide the disulfide bond closing which was ensured by the system of penalty potentials [50], possible values of the χ_1 angles of cysteine residues of about 60, 180, and –60° were sorted through.

In the third stage of the calculations, the data from the rotamer library for the side chain conformations were used [51] to optimize the side chain geometry at the prescribed values of the internal rotation angles calculated at the previous stage of the algorithm.

In the final stage, the three-dimensional structures of the fragment were energy optimized in an AMBER force field [52]. The energy was minimized using the conjugate gradient method [53].

Analysis of secondary structure elements. The data obtained for the internal rotation angles of the amino acid residues in the optimized structures were used to identify the regular secondary structure elements, β-turns, and unordered segments of the polypeptide chain.

To assign the residues to different types of the regular secondary structures, the following ranges of the angles ϕ, ψ [54] were considered:

$$\phi = -112.6 \pm 41.4°, \psi = 123.0 \pm 60°$$

for the residues with an elongated conformation;

$$\phi = -64.7 \pm 12.8°, \psi = -39.8 \pm 12.2°$$

for the residues in the right α-helix.

The β-turns were identified using the classification [55] and also the information about the specific interatomic distances $C^{\alpha}_{i}...C^{\alpha}_{i+3}$, calculated from the coordinates of the optimized structure atoms.

Comparative analysis of structures. The structures were compared using mean square deviations of the coordinates of the atoms and dihedral angles [45].

Because the precision (σ) of determination of the angles ϕ, ψ at the probability approach proposed in works [43-45] was 25° [56], the deviations over 75° (3σ) were considered significant for detecting reliable differences between the conformations of the amino acid residues [57].

To compare the structures, in addition to the mean square deviations, the χ^2 distribution [58] was also used to check the null hypothesis about the similitude of the variational series, at the significance $p = 0.99$.

RESULTS AND DISCUSSION

Secondary structure and spatial organization of the V3 loop in HIV-Haiti and HIV-MN virions. The table presents values of the dihedral angles ϕ, ψ, χ_1 of the amino acid residues of the HIV-Haiti and HIV-MN PND calculated by computer modeling on the basis of NMR spectroscopy data [22, 39] in water–TFE mixture.

Analysis of the dihedral angles of the HIV-Haiti V3 loop reveals on its N-end two elongated β-regions (residues 3, 4, and 8-14) separated by β-turn III (4-7). The C-terminal region of the loop forms an unordered structure (21-29), which on segment 31-35 changes to right α-helix conformation (Fig. 1). The central fragment of the HIV-Haiti V3 loop (residues 15-20) which forms the main immunogenic stretch of the HIV-1 [2] takes a triple β-turn IV-IV-IV conformation in the water–TFE mixture.

Analysis of the secondary structure in the HIV-MN strain shows (Fig. 1) that the variability of the amino acid composition of the V3 loop leads to a significant structural reorganization of the fragment. Thus, the 1-14 region of the V3 loop generating in the HIV-Haiti strain a clearly pronounced secondary structure in the HIV-MN is converted into an elongated irregular stretch (Fig. 1). Mutations in the primary structure of the gp120 protein also affect the C-terminal region of the analyzed fragment: in accordance with the calculated data (table), the 24-31 segment of the HIV-MN V3 loop forms a distorted α-helix that is absent in the correspondent PND segment of HIV-Haiti (Fig. 1). A similar situation also occurs in the case of a classical right α-helix 31-35 in the virus strain HIV-Haiti. These findings confirm the earlier noted by us tendency [30, 31] for production of coiled structures on the C-end of the V3 loop and are consistent with the results of studies on the HIV-1 PND peptides by NMR spectroscopy and CD approaches [24].

The comparison of the V3 loop secondary structure in the two strains of the virus has revealed a common structural element on the 15-20 segment: in the HIV-MN virion, similarly to HIV-Haiti, the main immunogenic stretch of the virus forms the non-standard triple β-turn in the water–TFE mixture (Fig. 1). This finding is

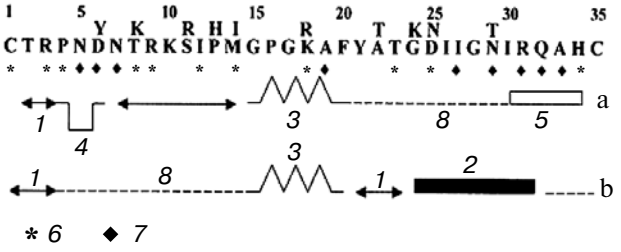

Fig. 1. Comparison of the V3 loop secondary structure elements in the virus strains HIV-Haiti (a) and HIV-MN (b) based on the NMR spectroscopy data [22, 39]: *1*) "elongated" conformation; *2*) distorted α-helix; *3*) triple β-turn IV-IV-IV; *4*) β-turn III; *5*) right α-helix; *6*) structurally conservative residues with virtually identical conformations in the two strains of the virus; *7*) conformationally stable amino acids located in the adjacent areas of the Ramachandran plot and specified by close values of the dihedral angles ϕ and ψ (table), with the difference between them not higher than 3σ; *8*) irregular conformation. At the top, the residues are shown which occupy the corresponding positions in the primary structure of the HIV-MN V3 loop.

Calculated values of dihedral angles for optimized three-dimensional structures of the HIV-Haiti and HIV-MN V3 loop

Residue	HIV-Haiti			HIV-MN		
	ϕ	ψ	χ_1	ϕ	ψ	χ_1
C-1	−72	−21	67	−77	−42	180
T-2	−67	−3	60	−84	130	52
R-3	−95	122	−169	−103	128	−160
P-4	−61	133	26	−53	133	30
N-5	−79	−5	−172	−58	−20	−176
D-6 (Y)	−83	−37	−173	−97	23	−170
N-7	−85	12	−168	−97	20	−177
T-8 (K)	−90	142	164	−100	135	−163
R-9	−85	140	−177	−102	128	−168
K-10	−108	168	−157	−85	16	−168
S-11(R)	−103	177	−174	−96	4	−163
I-12	−94	132	−167	−120	126	−172
P-13(H)	−44	144	27	−75	−17	−168
M-14(I)	−115	146	−171	−110	145	−177
G-15	−95	−46		98	−107	
P-16	−41	−26	29	−50	141	−25
G-17	117	4		99	−129	
K-18(R)	−102	30	−164	−104	9	−169
A-19	−67	−17		−69	−17	
F-20	−92	12	−171	−122	172	168
Y-21	−102	18	−177	−112	168	−178
A-22(T)	−71	−21		−93	127	52
T-23	−108	130	56	−103	141	56
G-24(K)	90	55		−84	−12	−172
D-25(N)	−80	−10	−169	−99	−7	−170
I-26	−107	147	−171	−56	−15	−167
I-27	−84	7	148	−59	−17	−159
G-28	86	29		−117	−4	
N-29(T)	−94	8	−171	−63	−26	53
I-30	−108	140	−163	−74	−3	−167
R-31	−84	13	−167	−90	−7	−171
Q-32	−87	−7	−160	−83	24	−169
A-33	−73	−8		−99	26	
H-34	−58	−8	−170	−65	−12	−160
C-35	−60	−30	72	−65	−20	64

Note: The amino acid sequence of the HIV-Haiti V3 loop is presented. The residues occupying the corresponding positions in the primary structure of the HIV-MN V3 loop are given in parentheses.

interesting in connection with data [36] on the double β-turn conformation realized on this region of the HIV-Haiti and HIV-MN V3 loop in aqueous solution. This conformation is similar to the structure detected by X-ray crystallographic analysis in the complexes of the V3 loop peptides with the Fab-fragments of the monoclonal antibodies 50.1 [16], 59.1 [17], and 83.1 [19]. The combined consideration of our findings with the data of [36] suggests that the HIV-1 IDE is a conformationally labile fragment sensitive to the environmental conditions: on addition of TFE, in both strains of the virus the double β-turn structure observed in aqueous solution [36] is transformed to the more compact spatial form of three overlapping turns of the polypeptide chain (Fig. 1). Note, that such an "architectural ensemble" was found in the V3 loop of the HIV-1 gp120 protein in 20% mixture of TFE–water [59].

The consideration of three-dimensional structures of the PND in the HIV-Haiti and HIV-MN strains shows (Fig. 2) that the immunogenic crown G-P-G-K/R-A-F

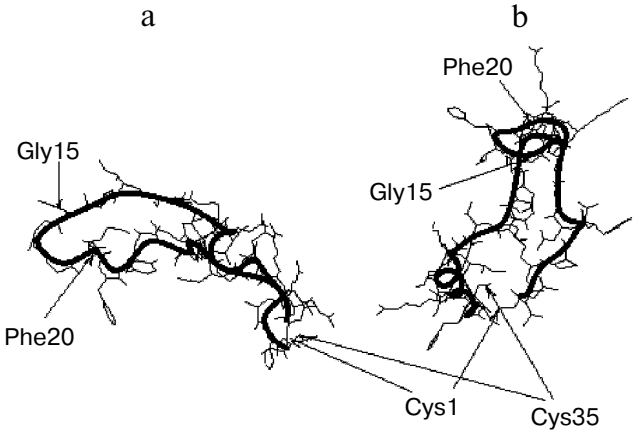

Fig. 2. Calculated three-dimensional structures of the gp120 protein V3 loop in HIV-Haiti (a) and HIV-MN (b) in TFE–water mixture. The calculation was performed using the Monte Carlo method + minimization [49] and force fields ECEPP2/3 [48] and AMBER [52]. To close the disulfide bond, a system of penalty potentials [50] was used. The side chain conformations were computed using the rotamer library [51]. The first and last residues of the G-P-G-K/R-A-F segment are indicated as well as the N- and C-terminal residues of cysteine.

of the V3 loop [2] also significantly contributes to the spatial packing of the fragment. Figure 2 shows that just these parts of the polypeptide chain provides in both structures the reverse turn resulting in the closing of the N- and C-terminal segments which include the majority of the residues involved in the cell tropism [60-63]. Certainly, this pattern found in the spatial structures of the V3 loop of both HIV-Haiti and HIV-MN strains is insufficient for a complete interpretation of their similitude degree in the HIV-1 virions under consideration. However, to solve our problem, we need reliable information about structurally conservative and labile regions of the V3 loop. Therefore, using methods of mathematical statistics, we have compared geometrical parameters of the calculated structures (Fig. 2) in the spaces of Cartesian coordinates of the atoms and dihedral angles.

Figure 3 presents the superposed structures of the main chain of the HIV-Haiti and HIV-MN V3 loop and its central part G-P-G-K/R-A-F, which forms the immunogenic crown of the virus [2]. Figure 3a shows significant discrepancies of the V3 loop spatial packing in the two strains of the virus confirmed by the mean square deviation of the atomic coordinates (6.3 Å). The comparison of the structures using the χ^2 test reveals systematic shifts between the variational series of their atomic coordinates.

These data show that the differences between the structures in the space of Cartesian coordinates are significant. Nevertheless, despite the significant structural discrepancies, the V3 loop of both the HIV-Haiti and HIV-MN strains produces in the water–TFE mixture the common structural element on the G-P-G-K/R-A-F segment (Fig. 3b). Thus, the mean square deviation of the main chain atom coordinates calculated for the structures presented in Fig. 3b equals 2.8 Å, which indicates a resemblance of the hexapeptide spatial forms in the HIV-Haiti and HIV-MN virions.

The structural characteristics of the main immunogenic stretch of HIV-1 were compared in the geometrical space of the internal rotation angles (table), and such three-dimensional structures in the hexapeptide package were concluded to originate from different local minima of the amino acid residues in its composition. This finding impressively supports the statement [31, 36] that the hexapeptide primary structure has a high reserve of "conformational strength" providing for similar spatial forms of the fragment in varied force fields. In other words, along with an extremely high lability of individual amino acids, the gp120 protein region under consideration is

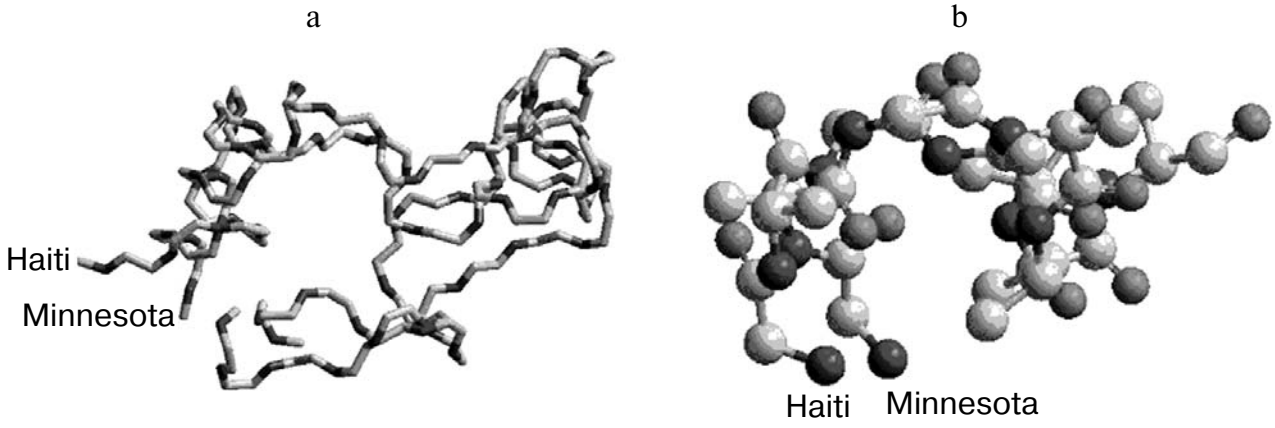

Fig. 3. Superposed structures of the main chain of V3 loop (a) and its principal immunogenic stretch G-P-G-K/R-A-F (b) in the virus strains HIV-Haiti and HIV-MN.

rather conservative in the geometrical space of the Cartesian coordinates of atoms, and this is manifested by a limited number of spatial forms of the main chain in the different HIV-1 virions [36].

The determination of structurally conservative amino acids of the HIV-1 V3 loop. The calculations using the computer modeling approaches have shown that, except for the HIV-1 immunogenic crown, the V3 loop of the HIV-Haiti and HIV-MN strains forms different secondary and tertiary structures in the water-TFE mixture (Figs. 1 and 3). This seems to be a consequence of conformational transitions of the fragment residues because of variability of its amino acid sequence. In fact, the comparison of the dihedral angles of the V3 loop in the HIV-Haiti and HIV-MN strains (Fig. 4) reveals considerable discrepancies between conformations of the individual residues: 15 amino acids undergo pronounced structural rearrangements, and the dispersion of the internal rotation angles suggests the significance of the differences between them. This conclusion is confirmed by calculations of the mean square deviations of the internal rotation angles for all residues of the fragment and its different segments. These results indicate that significant conformational changes concern both the terminal regions of the V3 loop and its main immunogenic part located in the central part of the HIV-1 PND. The mean square deviation of the angles calculated for all residues of the fragment equals 73.5° and is higher than the average value for the randomly chosen conformational states of the polypeptide chain [56]. Similarly to the above-considered case, fundamental discrepancies in the local structures are detected by the calculation of the variational series (ϕ, ψ), which reveals systematic shifts between the dihedral angles of the conformations compared.

We also conclude that more than 50% of the residues retain the conformational parameters in the HIV-Haiti and HIV-MN strains (Figs. 1 and 4). In this connection, a question arises about the degree of conformational lability of the amino acids constituting the functionally active stretches of the HIV-1 PND.

Only two residues (K/R and A) preserve the internal rotation angle values (Figs. 1 and 4) on the central G-P-G-K/R-A-F segment, which is involved in the overwhelming majority of contacts with the neutralizing antibodies [16]. For the other four amino acids, considerable discrepancies are observed which do not significantly change the spatial form of the hexapeptide (Fig. 3b). Among amino acids with changes in the conformation, the invariant residue P-16 should be noted. The dihedral angles of this residue, close to those presented in the table for the HIV-MN V3 loop in the water–TFE mixture, were obtained as a result of the investigation of three-dimensional structures of the virus main immunogenic stretch in the strains HIV-Thailand [34], HIV-MN [35], HIV-RF, and HIV-Haiti [36] in aqueous solution. In aqueous solution, the P-16 residue located in the B area of the space (ϕ, ψ) displayed a flexibility of the peptide chain manifested by structural transitions between two adjacent local minima, corresponding to the elongated conformation (HIV-MN and HIV-Haiti) and the M conformation (HIV-Thailand and HIV-RF) [36]. (The classification of the space areas (ϕ, ψ) and their determination based on the NMR spectroscopy data are described in [43]). The tendency of residue P for location in the B area of the conformational space is confirmed by X-ray crystallographic analysis of the V3 loop peptides bound with the antibodies 50.1, 59.1, and 83.1 [16, 17, 19]: in the above-mentioned structures values of its dihedral angles correspond to the prescribed area of the Ramachandran plot. However, the findings of the present work suggest that, under certain environmental conditions, the invariant proline residue can undergo pronounced structural transformations: according to the calculations (table), in the water–TFE mixture, the HIV-Haiti gp120 protein residue under consideration is located in the P area of the space (ϕ, ψ), and this correlates with the X-ray data on the structure of the V3 loop peptides bound with the monoclonal antibody 58.2 [18].

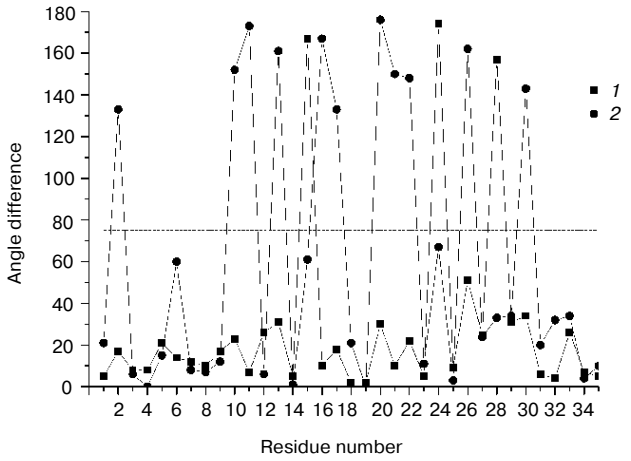

Fig. 4. Differences in the dihedral angles ϕ and ψ of the V3 loop amino acid residues in the HIV-Haiti and HIV-MN strains in the conformations adopted in water–TFE mixture: *1*) $\Delta\phi$; *2*) $\Delta\psi$. The horizontal line indicates the value of 3σ, which is used to reveal statistically significant differences between the local structures of the HIV-Haiti and HIV-MN V3 loops.

Thus, the conformational rearrangements of the viral IDE are initiated by the variability of the amino acid composition of the HIV-1 V3 loop, and this suggests the high sensitivity of the IDE local structure to changes in the force field of the fragment. This conclusion is consistent with the work [36] where the high lability of the amino acid residues constituting the fragment has been postulated based on the comparison of its conformations in aqueous solution in different strains of the virus. It seems that the conformational "mimicry" of the fragment which forms the main immunogenic stretch of the

HIV-1 can explain the specificity of the protein gp120 binding with the antibodies: the antibodies neutralizing virions of one strain of the HIV-1 can be inactive towards other viral particles [64].

Among amino acids that promote the penetration of the virus into the cell [6, 9-12, 63, 65], R-3, A-19, D/N-25, and Q-32 retain the local structure, whereas S/R-11, P/H-13, T-23, and G/K-24 manifest an increased conformational lability of the polypeptide chain (Fig. 4). Among structurally conservative amino acids, note the residue D/N-25 which determines the interaction of the virus with the primary receptor CD4 of the cell membrane [6, 9-12] and also the residue R-3 which plays a crucial role on the virus binding with the co-receptor CCR-5 [63, 65] and heparan sulfate proteoglycans [65]. The list of the conformationally stable amino acids, in addition to R-3, A-19, D/N-25, and Q-32, also includes residues 4, 8, and 33 occurring in the majority of the interpreted primary structures of the virions which use the co-receptor CCR-5 for penetration into the cell [63]. Mutations of the primary structure also do not considerably affect the conformation of residue 29. This finding is interesting in connection with the data of work [66] that the amino acid in the prescribed position of the V3 loop of the macaque monkey immunodeficiency virus is a component of the cell tropism determinant. The study on the C-terminal region of the HIV-1 V3 loop [67] has confirmed this conclusion: residue N-29 stabilizes its conformation and influences the intensity of the CD-4-activated gp120 protein binding with the co-receptor CCR5.

Among structurally conservative amino acids, the residues in positions 12 and 14 of the HIV-1 V3 loop should be also noted (Fig. 4), which significantly contribute to the interaction of the virus with the monoclonal antibody 447-52D [20, 68, 69] possessing a wide spectrum of neutralizing activity [70]. The structural stability of these residues seems, in particular, to explain the ability of the antibody 447-52D to neutralize different viral particles [70]. The investigation of effects of the amino acid substitutions on the cell fusion allowed the authors of work [71] to identify in the V3 loop of the HIV-1 B subtype six residues (positions 3, 6, 26, 29, 31, and 33) which were crucial for production of the syncytium. Five of these six residues retained the conformation in the virus strains under consideration (Fig. 4). The conformationally stable amino acids of the V3 loop are also supplemented with the segment residues 5-7 which include one of the possible sites of gp120 protein N-glycosylation [72], which is used by the virus for defense against neutralizing antibodies [73, 74] and elevation of infectivity [75-78].

Figures 1 and 4 show the non-uniform distribution of the structurally conservative amino acids along the polypeptide chain of the HIV-1 PND: the overwhelming fraction of the residues with the preserved conformations is concentrated on the N- and C-terminal regions which form the so-called stem of the V3 loop [60]. The central part of the loop, which forms the immunogenic crown of the virus [60], displays a pronounced conformational lability (Figs. 1 and 4). Obviously, the conservation of the conformational states of amino acids in the stem part of the HIV-1 V3 loop provides for the conditions necessary for the penetration of the virus into the cell, whereas the lability of the immunogenic crown helps the virus defense against neutralizing antibodies.

Thus, comparative analysis of the local structures of the V3 loop in HIV-Haiti and HIV-MN shows that a considerable fraction of the amino acids retains the conformations in these two strains of the virus. Obviously, these amino acids are promising targets for designing drugs for prevention and treatment of AIDS. However, researchers engaged in the antiviral drug design must pay attention not only to the conservative residues in the biologically active regions of the V3 loop but also to the residues with yet unknown functional role in the virus neutralization, cell tropism, and cell fusion. This is confirmed by results of the works [79-81] indicating the involvement of the V3 loop in formation of the neutralizing epitope, other than the main immunogenic region of the virus [79], and generation of the sites of the HIV-1 binding with the human saliva agglutinin [80] and heparan sulfate [65, 81].

The proposed model structurally supports the published experimental data on the biological activity of individual amino acid residues of the HIV-1 V3 loop and provides for a basis for studies on the interaction between the structure and function of the fragment, its dynamics, and the environment. Obviously, such information is necessary for creating a "cocktail" of immunogenic peptides, which is a promising candidate for the role of an antiviral vaccine [82, 83]. Such a cocktail has to contain as main components synthetic copies of the V3 loop fragments from different strains of the virus.

The work was supported by the Belorussian Republican Foundation for Basic Research (project X06-020).

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