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Conformational study of valinomycin: a molecular dynamics approach

S. Shobana, Saraswathi Vishveshwara *

Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India

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Abstract

Valinomycin is a highly flexible cyclic dodecadepsipeptide that transports ions across membranes. Such a flexibility in the conformation is required for its biological function since it has to encounter a variety of environments and liganding state. Exploration of conformational space of this molecule is therefore important and is one of the objectives of the present study that has been carried out by means of high temperature Molecular Dynamics. Further, the stability of the known bracelet-like structure of the uncomplexed valinomycin and the inherent flexibility around this structure has been investigated. The uncomplexed form of valinomycin has been simulated at 75–100 K for 1 ns in order to elucidate the average conformational properties. An alanine-analog of valinomycin has been simulated under identical conditions in order to evaluate the effect of sidechain on the conformational properties, The studies confirm the effect of sidechain on conformational equilibrium.

Keywords: Valinomycin; Alanine-analog; Molecular dynamics; Conformation search

1. Introduction

Ionophores bind to and carry ions across the membranes. Some of them are cyclic peptides or depsipeptides. Its functional role demands an understanding of the equilibrium conformational properties and the dynamics of the molecule. An up-to-date efforts on the conformational properties of various peptides are summarized by Hruby [1]. The present study is carried out on the depsipeptide, valinomycin using Molecular Dynamics (MD) method.

Valinomycin is an ionophore that has been extensively studied for its ion binding capacity, conformational properties and its ability to transport ions across membranes. It can adopt a variety of conformations and the current interest lies in exploring the conformational space and correlating the conformation of valinomycin with its different phases of ion-binding and transporting activities. In the present study, we have attempted to explore the conformational space of uncomplexed valinomycin by high temperature MD studies. The uncomplexed valinomycin has also been studied at different lower temperatures. Further, the specificity of valinomycin is known to be altered when the sidechain residues are replaced by other groups. In order to assess the effect

* Corresponding author.

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of sidechains on the conformational preferences, the uncomplexed valinomycin and its alanine analog have been subjected to 1-ns MD simulation at 75–100 K. The average conformation, the conformational transitions and the effect of sidechains have been discussed based on the results of MD studies.

A brief review of conformational studies on uncomplexed valinomycin is presented in this paragraph. Valinomycin being an ionophore has been extensively studied by various experimental techniques [2–10] and theoretical methods [11]. The molecule is a cyclic dodecadepsipeptide (L-Val–D-Hiv–D-Val–L-Lac)$_3$ and contains six amide linkages, six ester linkages and side chain of hydrophobic alkyl groups. A large number of conformational possibilities are predicted for this molecule. Solution studies [5,6] proposed three conformational models depending on the nature of the solvent: A (bracelet structure), B (propeller structure) and C (open structure) differing in the number of intramolecular hydrogen bonds. In non-polar solvents it is known to predominantly exist in conformation A, which has six intramolecular 4 → 1 hydrogen bonds while in medium polar solvents, it exists in form B containing 3 intramolecular 4 → 1 hydrogen bonds and in highly polar solvents it exists in form C containing no intramolecular hydrogen bonds. The uncomplexed molecule crystallized from non-polar solvents has a pseudosymmetric center and consists of six intramolecular hydrogen bonds, of which four are 4 → 1 type while the other two are 5 → 1 type of hydrogen bond [12–14]. The crystal structure obtained by Karle et al. [15] has the propeller form (form B) in DMSO. A number of ion complexes of valinomycin have also been well characterized (see reviews [3,11,16] and references cited therein) which also confirm the fact that valinomycin is an extremely flexible molecule that can adopt varied conformations depending on its environment. MD studies being a powerful tool can access a large number of conformations and can elucidate the flexibility inherent to this molecule in a quantitative way. Recently, high temperature MD studies in vacuum have been employed on related polycyclic molecules for conformational sampling [17,18]. To the best of our knowledge, the only report on MD studies in the lipid environment has been by Edholm [19] on glycophorins while the study on the ionophore, nonactin, by Marrone and Merz [20] has been carried out in methanol.

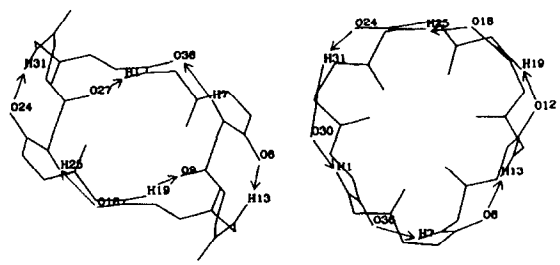
2. Methods

2.1. MD procedure

The current MD studies on valinomycin were carried out on an INTEL 860 machine using AMBER [21,22] in which the potential function describing the interaction of the system has the following form:

$$E_{\text{total}} = \Sigma_{\text{bonds}} K_R (R - R_0)^2 + \Sigma_{\text{angles}} K_\theta (\theta - \theta_0)^2 + \Sigma_{\text{dihedrals}} V_n/2[1 + \cos(n\phi - \gamma)] + \Sigma_{i<j \text{non-bonded}} \left[B_{ij}/(R_{ij})^{12} - A_{ij}/(R_{ij})^6 + q_i q_j/\epsilon R_{ij} \right] + \Sigma_{\text{H-bonds}} \left[C_{ij}/(R_{ij})^{12} - D_{ij}/(R_{ij})^{10} \right]$$

The AMBER [23,24] all atom force field parameters supplemented with parameters generated for ester linkages [25] and ab initio partial charges [26,27] were used. To avoid *cis–trans* isomerization at high temperature the barrier for the peptide bond was increased [28] by 10 kcal/mole. SHAKE was applied to constrain all the carbon–hydrogen bond lengths. The simulation was performed with a distance dependent dielectric constant $\epsilon = 1r$, which



(a) (b)

Fig. 1. Conformation of valinomycin as in (a) uncomplexed crystal structure (b) K^+-complexed structure without the ion. The sidechains are not indicated. The arrows indicate the six hydrogen bonds present. The numbering scheme is given in Fig. 2.

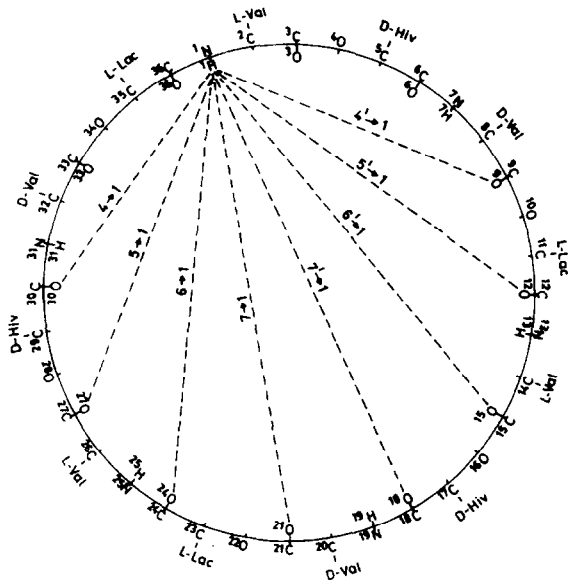


Fig. 2. A schematic representation of valinomycin indicating all possible hydrogen bonds with respect to ^{1}N–H. The backbone atoms are numbered from 1 to 36. The numbers on amide hydrogens and carbonyl oxygens are the same as the backbone amide nitrogen and carbonyl carbon, respectively.

takes into account the screening due to polarization of the atoms of the solute and it helps to compensate for the lack of explicit solvent [23]. The time step used was 0.001 ps. The coordinates were saved at every 0.1 ps.

In order to ensure that the conformational sampling has no bias towards the starting conformation, two crystal structures were chosen for the high temperature studies (Fig. 1). They include the pseudosymmetric crystal structure with four 4 → 1 and two 5 → 1 intramolecular hydrogen bonds [13] and the bracelet structure with a three-fold symmetry with six 4 → 1 intramolecular hydrogen bonds (K^{+}-complex like structure) [10], where the ion has been excluded. The structures were minimized energetically until the gradient was less than 0.001 kcal/mole Å. The simulations were carried out at 900 K for a period of 100 ps. The 2000 sample points (1000 from each of the simulations) were then minimized to an energy gradient less than 0.01 kcal/mole Å and were then subjected to various analyses.

The starting structure for the 1-ns simulations on valinomycin is the uncomplexed pseudosymmetric crystal structure [13] with four 4 → 1 and two 5 → 1 hydrogen bonds. The alanine analog of valinomycin has been obtained by changing all the sidechain residues of valinomycin to alanine and is further referred to as Ala-valinomycin in this report.

2.2. MD analysis

Hydrogen bond

It has been found that the best method to characterize the shape and conformation of a cyclic peptide like valinomycin is through the intramolecular hydrogen bonds. Though, 4 → 1 and 5 → 1 type of hydrogen bonds are generally reported in the various studies on valinomycin, the molecule with its six amide protons, six amide carbonyls and six ester carbonyls in principle, can have a variety of hydro-

Table 1
48 possible hydrogen bonds involved in valinomycin [a]

Proton donors	Proton acceptors							
	Type of hydrogen bond (N...O)							
	4 → 1	5 → 1	6 → 1	7 → 1	7′ → 1	6′ → 1	5′ → 1	4′ → 1
N31	O24(1) [b]	O21(7)	O18(13)	O15(19)	O12(25)	O9(31)	O6(37)	O3(43)
N25	O18(2)	O15(8)	O12(14)	O9(20)	O6(26)	O3(32)	O36(38)	O33(44)
N19	O12(3)	O9(9)	O6(15)	O3(21)	O36(27)	O33(33)	O30(39)	O27(45)
N13	O6(4)	O3(10)	O36(16)	O33(22)	O30(28)	O27(34)	O24(40)	O21(46)
N7	O36(5)	O33(11)	O30(17)	O27(23)	O24(29)	O21(35)	O18(41)	O15(47)
N1	O30(6)	O27(12)	O24(18)	O21(24)	O18(30)	O15(36)	O12(48)	O9(48)

[a] The numbering scheme of the proton donors and acceptors are given in Fig. 2.
[b] The hydrogen bonds between the different proton donors and acceptors are assigned the numbers given within the brackets.

gen bonds with varying numbers. Every single amide proton can have hydrogen bond interaction with eight carbonyl oxygen of the ring (neglecting the nearest neighbors) which is schematically represented in Fig. 2. Thus, valinomycin in principle, can have 48 intramolecular hydrogen bond interactions (greater than 3 → 1 interactions) as given in Table 1. Hence, apart from the predominantly observed 4 → 1 and 5 → 1 type of hydrogen bonds, the other possible hydrogen bonds are 6 → 1, 7 → 1, 7′ → 1, 6′ → 1, 5′ → 1 and 4′ → 1 as shown in Fig. 2. 6 → 1 type of hydrogen bond has been identified in the crystal structure of the D,L stereoisomeric analog of valinomycin [29]. A distance criterion of d(H...O) ≤ 2.3 Å and angle criterion of 180° ≤ angle N–H...0 ≤ 155° are chosen for analysis.

Dihedral angles

The conformation of valinomycin is also expressed in terms of the backbone dihedral angles of the cyclic structure and they were assigned as '+' (gauche$^+$: +60° ± 20°), '−' (gauche$^-$: −60° ± 20°), 't' (anti: 180° ± 20°). The other conformations were further categorized as follows: '1' (+90° ± 10°), '2' (+120° ± 20°), '3' (+150° ± 10°), '4' (−90° ± 10°), '5' (−120° ± 20°), '6' (−150° ± 10°), '7' (+30° ± 10°), '8' (0° ± 20°) and '9' (−30° ± 10°) to represent the entire conformational range and to facilitate the analysis of the varied conformers obtained.

The trajectories and the diagrams of the molecules were generated by using InsightII version 2.3.5 [30].

3. Results and discussion

3.1. Conformational search by high temperature MD

The 2000 MD minimized structures obtained from the two simulations were analyzed by means of hydrogen bond interactions and ring dihedrals and some of these structures were tested for their stability by simulating further at 100 K for 100 ps and were found to be stable. The energies of the minimized crystal structures are −33 and −28 kcal/mole respectively). The energies of 30 lowest conformations ranged from −32 to −22 kcal/mole. However, in these structures the energies are not correlated with the number of hydrogen bonds. The small energy difference between largely different conformations of valinomycin indicates that it can easily adopt varied conformations. It is likely that the

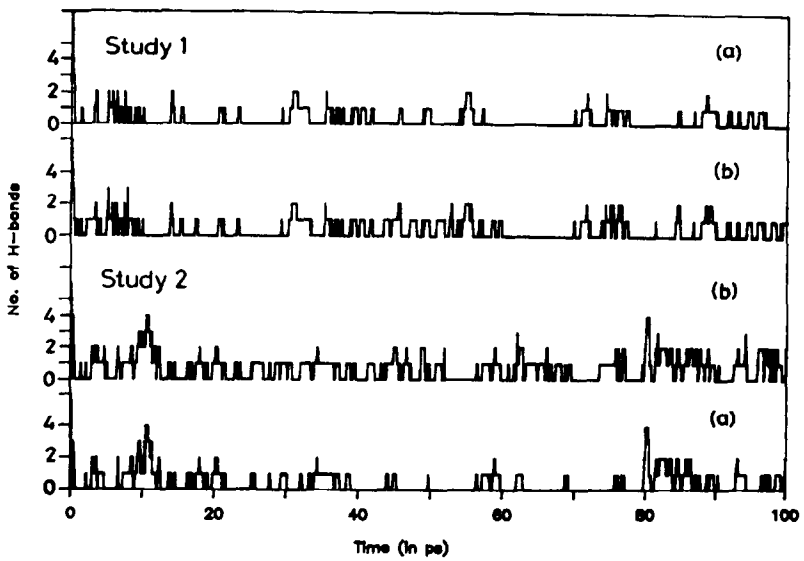


Fig. 3. A plot of the number of hydrogen bonds in the MD minimized structures over a period of 100 ps. Only 4 → 1 and 5 → 1 hydrogen bonds are included in graph (a) and all type of hydrogen bonds are included in graph (b).

Table 2
Structures containing hydrogen bonding distances obtained from study (1) and (2) of uncomplexed valinomycin [a]

Structure type	Total no. of	Total no. of	Other types of H bonds [b]
1	4+2=6	1	
2	6+0=6	1	
3	5+1=6	2	
4	5+0=5	1	
5	4+1=5	1	
6	4+0=4	1	
7	3+1=4	2	1; 7 → 1
8	3+0=3	1	
9	2+1=3	4	1; 4′ → 1
10	2+0=2	11	1; 4′ → 1 2; 7′ → 1 1; 7 → 1 and 7′ → 1
11	1+1=2	1	
12	1+0=1	8	5; 7 → 1 1; 4′ → 1 2; 7′ → 1
13	0+0=0	12	(i) 4; 7 → 1; 7′ → 1 (ii) 2; 4′ → 1 (iii) 1; 6 → 1; 6′ → 1; 7′ → 1

[a] In this and subsequent tables, study (1) and study (2) refer to high temperature MD for structures 1 and 2 as the starting points respectively. [b] Some of the structures listed have distances other than 4 → 1 and 5 → 1 type. The types of distances found in those structures are given in this column.

ligand and the environment has a major role to play on the conformation adopted by valinomycin.

Hydrogen bond analysis

Fig. 3 gives the pictorial representation of the hydrogen bond analysis of the 1000 structures from study 1 and 2. When all the 48 types are included for computing the total number of hydrogen bonds present in a structure, the conformers containing such hydrogen bonds increase by a considerable number in contrast to the case when only the 4 → 1 and 5 → 1 types are considered. The analysis showed about 40 structures with at least one hydrogen bond. They are classified into 13 different types which are listed in Table 2. It is clearly evident from the table that the more compact structures consisting of significant number of 4 → 1 and 5 → 1 type of hydrogen bonds are few in number while the more open forms with abundance of other types of hydrogen bonds are more in number. The shapes of these conformers can be better perceived from Fig. 4a and Fig. 4b. Fig. 4a gives the more compact structures with more of 4 → 1 and 5 → 1 type of hydrogen bonds while Fig. 4b the more open structures which have more of the 6 → 1, 7 → 1, 7′ → 1, 6′ → 1, 5′ → 1 and 4′ → 1 type of hydrogen bonds.

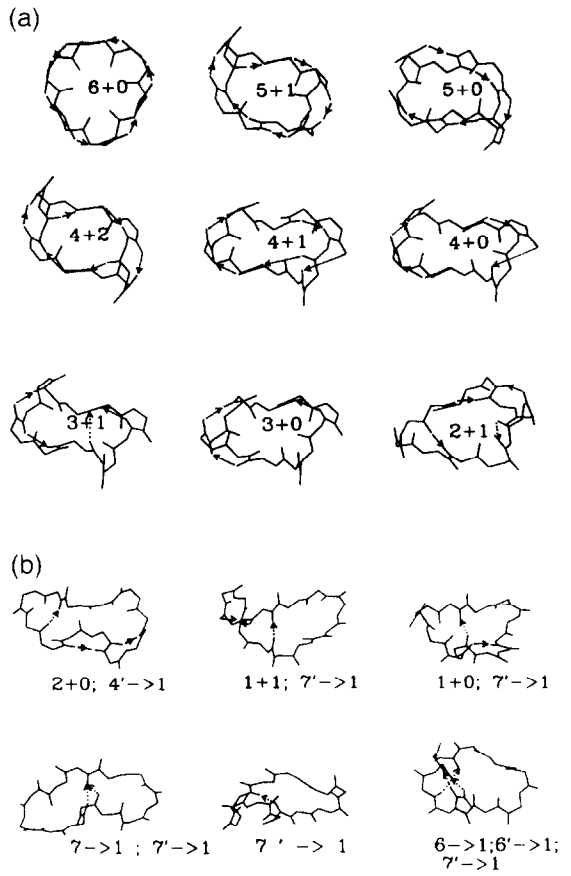


Fig. 4. (a) Schematic diagram of structures 1–9 given in Table 2. The numbers within the molecular plot represent the number of 4 → 1 + 5 → 1 hydrogen bonds which are represented by solid arrows. The dotted arrows represent the hydrogen bonds other than the above mentioned types. (b) Schematic diagram of some of the structures from 10–13 given in Table 2. The numbers below the molecular plot represent the number of 4 → 1 + 5 → 1 hydrogen bonds and the other type of hydrogen bonds present in the structure. The 4 → 1 and 5 → 1 hydrogen bonds are represented by solid arrows. The dotted arrows represent the hydrogen bonds other than the above mentioned types.

Dihedral angle analysis

The dihedral angles corresponding to the two minimized structures chosen for high temperature MD as well as for some of the hydrogen onded structures listed in Table 2 are given in Table 3. The ϕ values taken by all the conformers listed in Table 3 reflect the chirality of the residues i.e., the ϕ values of L-residues are generally in the '−' region and those of D-residues are in the '+' region. However, ϕ values are occasionally found in the 't' region in the case of D-Hiv and L-Lac. The ψ values are generally found to adopt conformations spreading over the entire 360°. The frequency and percentage of occurrences of various sets of (ϕ,ψ,ω) obtained by the analysis of 2000 minimized structures are given in Table 4, which clearly indicates the preference of those sets of (ϕ,ψ,ω) that are present in the initial conformation.

Table 4
The total occurrences and the percentage of high frequency (ϕ,ψ,ω) sets in the two simulations

Study (1)			Study (2)		
Type	Total	%	Type	Total	%
+7t	799	6.67	+7t	1013	8.44
++t	652	5.43	−−t	609	5.07
−2t	473	3.94	++t	511	4.26
−−t	450	3.75	−+t	486	4.05
+5t	386	3.22	−2t	417	3.47
−+t	356	2.97	+5t	406	3.38
−3t	338	2.82	−9t	377	3.14
−tt	301	2.51	37t	320	2.67
t7t	289	2.41	+6t	299	2.49
+6t	284	2.37	+−t	284	2.37
−9t	259	2.16	−3t	247	2.06
+−t	241	2.01	t7t	247	2.06

To carefully examine the preferred conformations of the four kinds of residues in the two simulations, the $(\phi$–$\psi)$ plot corresponding to the Ramachandran map [31] were obtained and were then compared with the standard ϕ-ψ maps [32]. Fig. 5 gives the ϕ–ψ contact map for a dipeptide (PP), ester-peptide (EP) and peptide-ester (PE) units having L-alanyl residue at the junction. The differences between the three maps are the following: (i) in the EP plot, the left handed α-helical region is found to be not allowed unlike in PP and PE (region C in III quadrant); (ii) in the plot the bridge region for PP indicates that the region $\phi = -140°$ to $-180°$, $\psi = 0° \pm 30°$ is disallowed while the maps of EP indicates is allowed and PE indicates the region $\phi = 0° \pm 20°$ is disallowed; (iii) the allowed regions with normal limits are restricted to ϕ between $\cong 55°$ and $\cong 95°$ in EP whereas it is broader (ϕ: $\cong 55°$ to $\cong 155°$) in PP and PE.

In Fig. 6, the (ϕ,ψ) plot corresponding to each

Table 3
The backbone dihedral angles (ϕ,ψ,ω) of valinomycin with varying number of hydrogen bonds

Hydrogen bonded structure [a]	Backbone dihedral angles (ϕ,ψ,ω)											
1 [b]	−2t	+8t	+5t	−8t	4+t	t−t	+5t	−8t	−2t	+8t	1−t	t+t
2 [b]	−2t	+7t	+5t	−8t	−2t	+7t	+5t	−8t	−2t	+7t	+5t	−8t
3	−2t	+7t	+5t	−9t	−1t	38t	+5t	48t	−2t	+7t	1−t	t+t
4	−2t	+7t	1−t	t+t	−2t	+8t	+5t	−9t	−1t	39t	+5t	48t
5	−2t	+8t	15t	4+t	63t	+−t	15t	−9t	−1t	+8t	16t	4+t
6	−2t	+7t	15t	4+t	63t	1−t	+5t	−9t	−1t	+8t	+6t	4+t
7	−2t	+7t	15t	4+t	63t	1−t	+5t	−9t	−1t	+8t	+6t	4+t
8	−2t	+8t	15t	4+t	63t	+−t	15t	−9t	−1t	+8t	+tt	4+t
9	−2t	+7t	+53	t+t	−3t	+7t	+4t	t+t	−3t	+−t	353	−9t
10	51t	+7t	26t	−9t	−2t	3−t	+−t	t9t	12t	+−t	26t	−2t
11	51t	+6t	153	−tt	536	++t	2+t	−3t	3t6	+1t	14t	t+t
12	−−3	+7t	2−6	t8t	++6	37t	27t	−3t	33t	+7t	+tt	+5t
13(i)	−−t	−4t	+5t	−−t	5−t	+6t	28t	−1t	−2t	++t	t6t	−1t
13(ii)	62t	+7t	25t	t4t	6tt	++t	2−3	−tt	38t	−9t	++t	+7t
13(iii)	58t	t9t	292	−28	6+t	+−t	75t	t7t	1−t	68t	t5t	−−8

[a] Details of the hydrogen bonded structures are given in Table 2. [b] Structure 1 and 2 correspond to the starting structures of study (1) and (2), respectively.

residue obtained from 1000 MD minimized structures from study 1 are plotted (only that of study 1 is given, since it was very much similar to that of study 2). They are superposed on their respective standard maps. The differences found between the standard maps and those obtained from the high temperature studies are minor. The spread around the allowed regions of ϕ is narrow in the case of L-Lac and D-Hiv (EP) compared to L-Val and D-Val (PE). The other noticeable difference is between the L- and D-maps of the same class which reflects the chirality of the residue. Further in the case of L-Val and D-val, the populations in the different quadrants are found to be different particularly, in the IV quadrant. In the case of D-Hiv, the III quadrant is more populated than IV unlike L-Lac where IV is more populated than III. Thus, the maps obtained from the high temperature studies and the standard maps are comparable in general. The minor differences can be attributed to various factors namely, the differences in the nature of sidechain, the neighboring residues and the ring closure constraint. It should also be noted that the standard maps are drawn for constant bond angles ($\tau = N - C^{\alpha} - C^{\beta}$) whereas in the case of MD minimized structures the bond angles are allowed to vary.

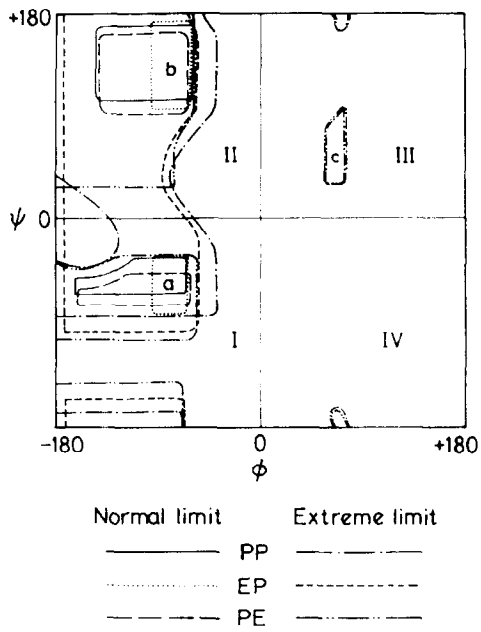


Fig. 5. The (ϕ - ψ) contact maps of L-alanine dipeptide (PP), ester-peptide (EP) and peptide-ester (PE) are superposed. The regions a, b and c represent the standard right-handed α-helical, extended and left-handed α-helical region. The map is divided into four quadrants I, II, III and IV.

3.2. Comparison of valinomycin and Ala-valinomycin simulations

Uncomplexed valinomycin simulation

MD simulations at 300 K showed that the initial conformation such as the bracelet structure of the uncomplexed valinomycin is not highly stable as indicated by the disruption of the intramolecular hydrogen bonds which are shown in Fig. 7. Such an unstable behavior at temperatures close to 300 K were observed by Forester et al. [33], Eisenman et al. [34], who could reproduce the experimentally observed ion selectivity of valinomycin at 100 K and which could perhaps be the effect of not including the environment. Hence simulations were performed at lower temperatures of 250 K, 200 K, 150 K, 100 K and 50 K. These simulations followed by the hydrogen bond analysis (Fig. 7) for their hydrogen bond stability showed that a temperature around 75 K was suitable for extended simulation of 1-ns. Hence the uncomplexed valinomycin and its alanine analog were subjected to 1-ns MD simulation at about 75–100 K.

The trajectory analysis of 48 distances mentioned in Table 1 were carried out and the 4 → 1 and 5 → 1 types of hydrogen bonds are presented in Fig. 8a and Fig. 8b respectively. The trajectories indicate two major transitions (i) between 77 and 82 ps and (ii) between 634 and 638 ps. The starting structure has four 4 → 1 type of hydrogen bonds (1, 2, 4 and 5) and two 5 → 1 type of hydrogen bonds (9 and 12). During the first transition, it changes to five 4 → 1 type of hydrogen bonds (1, 2, 3, 4 and 5) and one 5 → 1 type of hydrogen bond (12). The structure in the region 85 to 630 ps with five (4 → 1) and one (5 → 1) hydrogen bond is designated as structure-1 (ST1) and the structure in the region 640 to 1000 ps as structure-2 (ST2), which is same as the starting point.

From Fig. 8a and Fig. 8b, it can be seen that the major change between the two structures is restricted to the distance '3', a 4 → 1 hydrogen bond and the

distance '9', which is a 5 → 1 hydrogen bond between corresponding atoms. Analysis of distance trajectories revealed that the four distances '21' and '24' (7 → 1 type), '35' (6 → 1 type) and '48' (4′ → 1 type) also exhibit noticeable fluctuations during the interconversion of ST1 and ST2 and vice versa. These trajectories are presented in Fig. 8c.

During the interconversion of ST1 ⇔ ST2, the major conversion that occurs is between the pair of 4 → 1 and 5 → 1 hydrogen bonding distances 3 and 9. The mechanism of ion capture envisioned by Pullman [35] and Duax [36] implies that one of this kind of pair of 4 → 1 and its corresponding 5 → 1 hydrogen bonds could possibly initiate the capture of

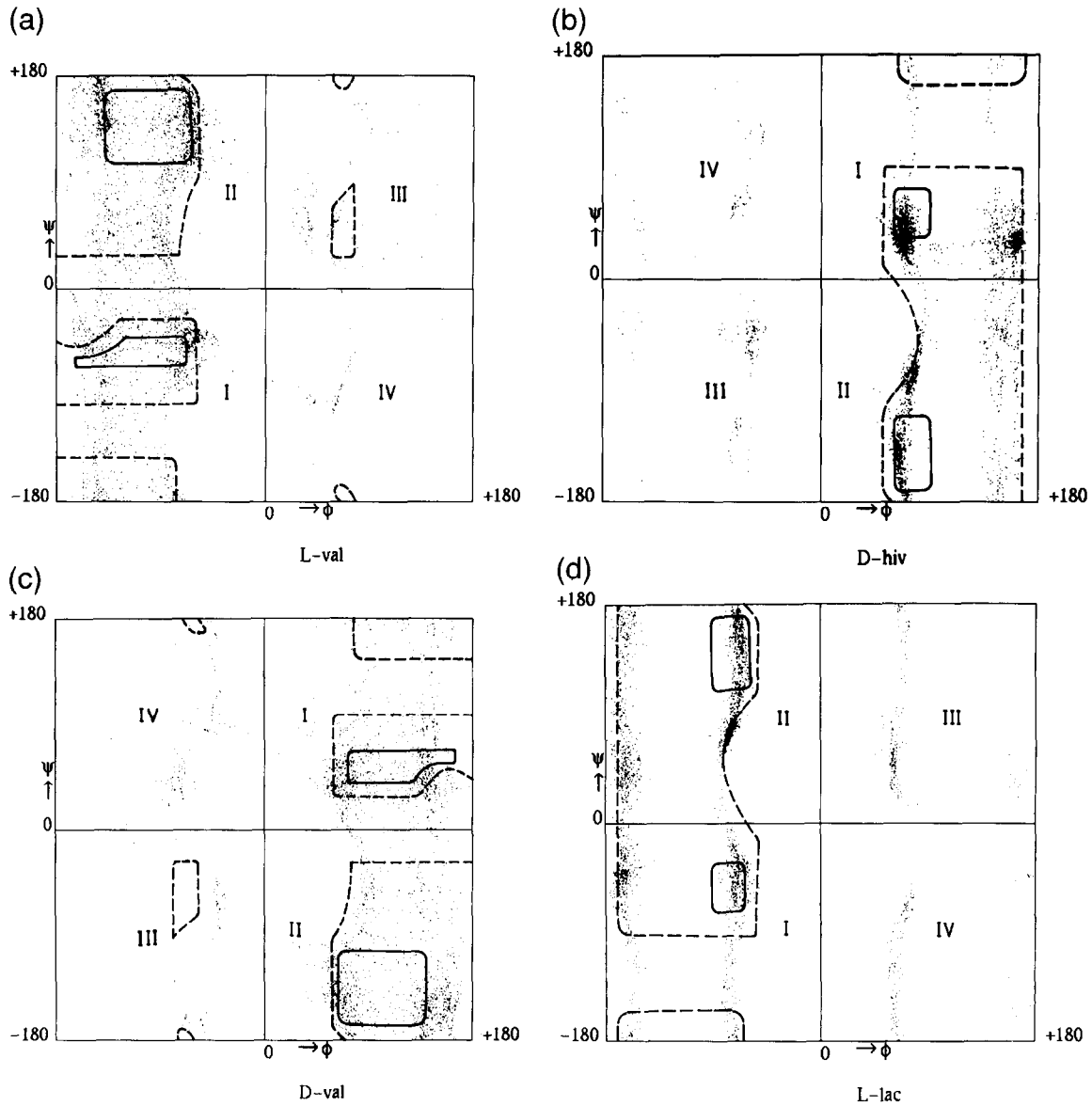


Fig. 6. The (ϕ,ψ) diagrams of (a) L-Val, (b) D-Hiv, (c) D-Val, (d) L-Lac segments of valinomycin. The points are obtained from the MD minimized structures of study 1. The respective standard allowed maps presented in Fig. 6 are superposed on these points. (–) represents the normal limit and (- –) the extreme limit.

the cation, thereby leading to the rupture of 5 → 1 hydrogen bonds and consequential changes in the bracelet like conformation. This proposal is being supplemented by ST1 obtained from our studies which is similar to the crystal structure of the analog isoleucinomycin [37]. Thus to gain insight into this initial structure of the ion capture mechanism, the transitions are probed in more detail. A study of energetics of the entire simulation does not exhibit any observable change in the potential energy during transitions. Also, the energy difference between the minimized ST1 and ST2 is found to be negligible where the energy at the transition point is about 2.0 kcal/mole relative to ST1 and ST2. Hence, the interconversion of the two structures is energetically facile with a small barrier. This is concurrent with the analysis of by Pletnev et al. [37] which suggests that the energy barriers to the transitions between the various conformers of valinomycin and its analogs found in the crystalline state are very small.

Ala-valinomycin simulation. The hydrogen bond trajectories of Ala-valinomycin are presented in Fig. 9a and Fig. 9b. It is interesting to note that Ala-valinomycin prefers to stay essentially in ST2 (four 4 → 1 and two 5 → 1 hydrogen bonded structure) unlike valinomycin. It can be seen from Fig. 9a and Fig. 9b that the starting structure (ST2) with four

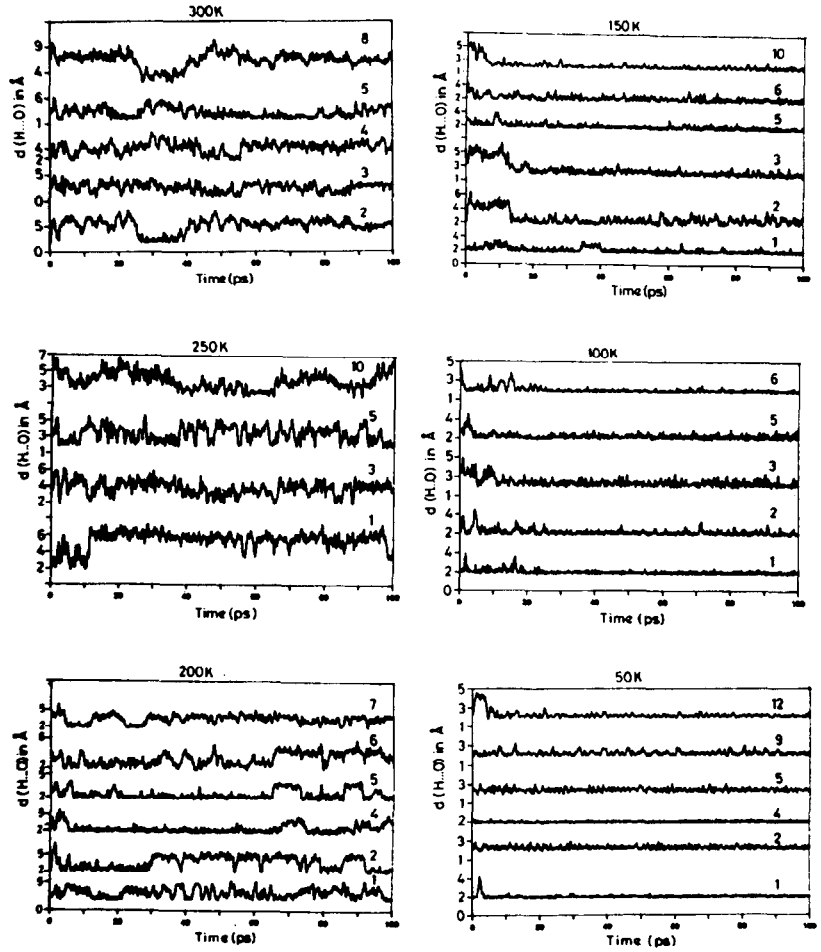


Fig. 7. The hydrogen bond trajectories of valinomycin simulated at (a) 300 K, (b) 250 K, (c) 200 K, (d) 150 K, (e) 100 K and (f) 50 K. The numbers as inset on the trajectories indicate the 4 → 1 and 5 → 1 hydrogen bond between atoms given in Table 1.

4 → 1 and two 5 → 1 hydrogen bonds is remarkably stable throughout most of the simulation period. It changes to ST1 with five 4 → 1 and one 5 → 1 hydrogen bonds only for a very brief period during 940 to 960 ps. During this time the hydrogen bond '6' (4 → 1) is gained with corresponding loss in the

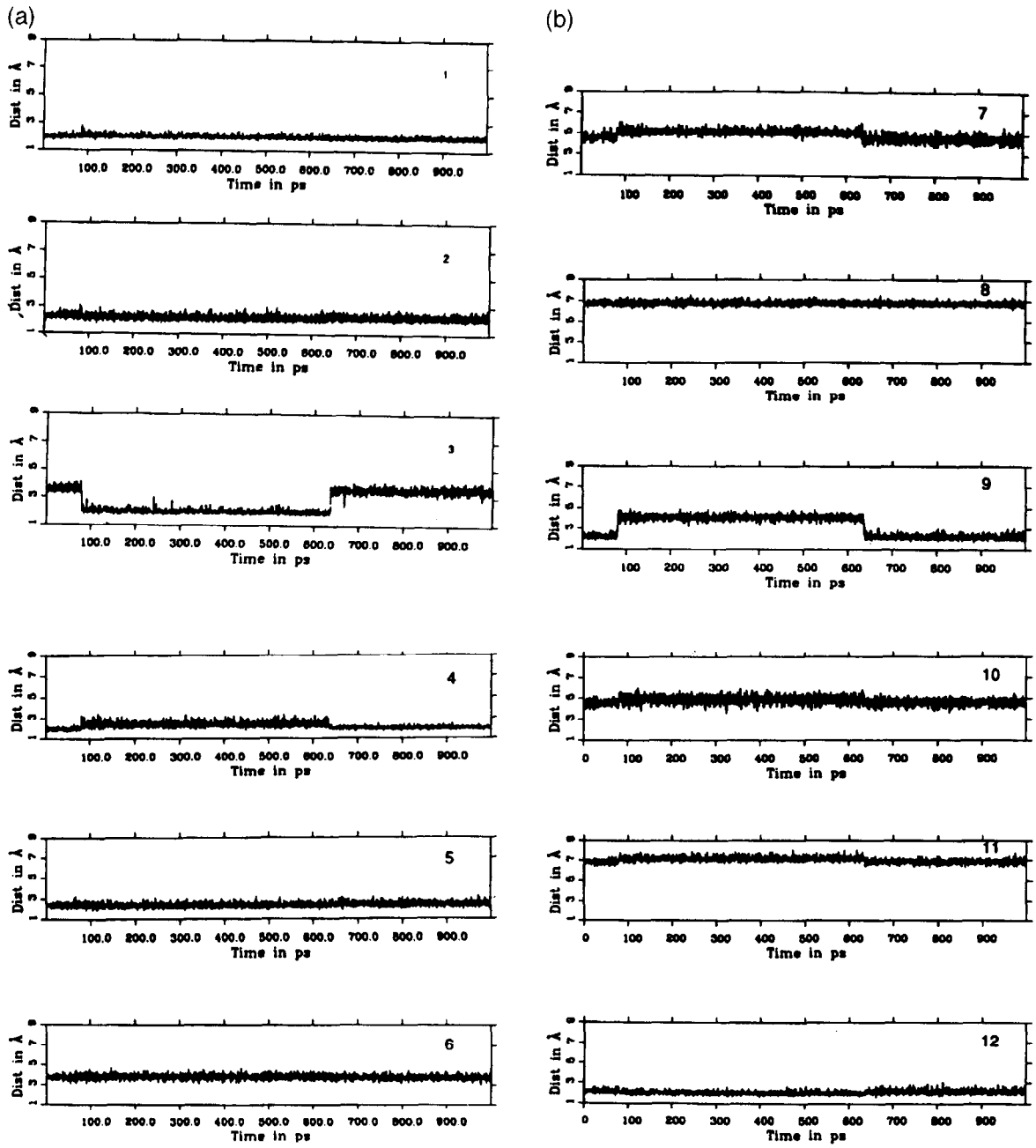


Fig. 8. The MD trajectories of hydrogen bonding distances. (a) 4 → 1 type, (b) 5 → 1 type, (c) other types (7 → 1, 6 → 1 and 4′ → 1) of the 75 K simulation with uncomplexed valinomycin as the initial conformation. The numbers printed as inset in the trajectories correspond to the hydrogen bonding distance number as given in bold in Table 1.

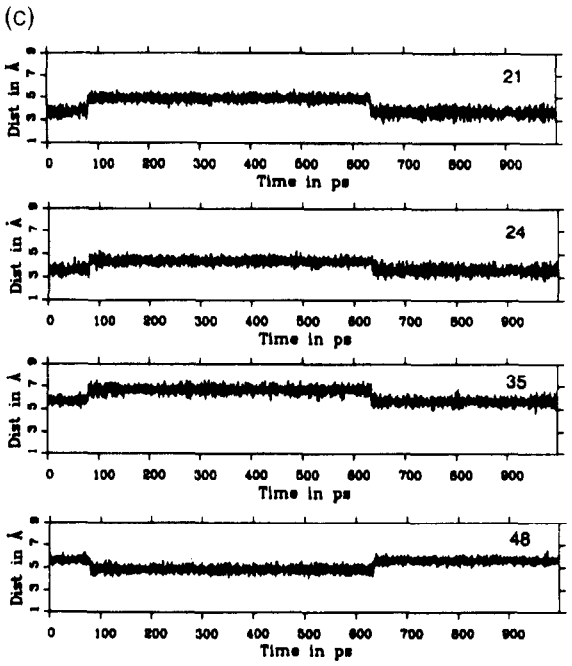


Fig. 8 (continued).

5 → 1 hydrogen bond '12'. The transition is also accompanied by minor variations in the 4 → 1 and 5 → 1 type distances namely, '1', '2', '8' and '10' and a few other 6 → 1, 6′ → 1, 7 → 1, 7′ → 1 and 4′ → 1 interactions. Two reasons can be offered for the preference of symmetric ST2 for alanine analog: (1) since all residues are alanine, the molecule might have a high preference for a totally symmetric structure OR; (2) in valinomycin, when ST1 is attained, the bulky hydrophobic groups resembling the membrane environment may prevent the immediate transition to ST2.

In summary, the present MD simulations reveal that valinomycin has more conformational flexibility than Ala-valinomycin under a given condition.

4. Conclusions

The conformational space of uncomplexed valinomycin has been explored by MD at 900 K, followed by energy minimization. The resulting structures are analyzed in terms of 48 types of intramolecular hydrogen bonds and backbone dihedral angles. The results indicate that apart from the known 4 → 1 and 5 → 1 type of hydrogen bonds, structures with hydrogen bonds of the type 6 → 1, 7 → 1, 5′ → 1, 6′ → 1 and 7′ → 1 have also been obtained giving rise to a variety of shapes for valinomycin molecule.

The dihedral angle analysis of the points obtained by high temperature MD-minimization revealed that the (ϕ,ψ) maps of D-Hiv and L-Lac (EP) and D-Val and L-Val (PE) are found to be similar to that of dipeptide with many of the points falling in the α-helical and β-sheet regions. The ϕ values are more localized than ψ and the distribution of points in different regions are generally found in the allowed regions of the map and is slightly influenced by factors such as the nature of sidechain and the nature of neighboring residues. The ring closure constraint has not significantly influenced the allowed regions in the (ϕ,ψ) map.

Simulations of uncomplexed valinomycin between temperatures of 300 K and 50 K showed that detailed study of properties can be made between temperatures of 75–100 K. It is likely that inclusion of explicit solvent molecules could overcome the shortcomings of the simulations of the isolated molecule.

From the one-nanosecond Molecular Dynamics studies on valinomycin and its alanine analog with the crystal structure of valinomycin as the starting geometry, the following conclusions can be drawn. (a) The nanosecond simulation of the crystal structure of the uncomplexed valinomycin showed that two conformations are in equilibrium namely, the crystal structure (ST2) is identified by four (4 → 1) type and two (5 → 1) type of hydrogen bonds. (b) ST1 has five (4 → 1) and one (5 → 1) hydrogen bonds as seen in the crystal structure of a modified valinomycin, isoleucinomycin — a conformation which has been implicated as a stable intermediate in the pathway related to ion transport [37]. The mechanism of interconversion of these two structures by virtue of the make and break of 4 → 1 and 5 → 1 hydrogen bonds accompanied by the changes in other distances have been characterized.

Substitution of all the sidechains by alanine residues shows that the equilibrium conformation corresponds only to ST2. It indicates that the sidechains have a role to play in controlling the

population of different conformation under equilibrium conditions. These results provide evidence for the conformational flexibility and sensitivity of the valinomycin backbone to small changes in stereochemistry of the sidechains. This observed conformational difference between valinomycin and its

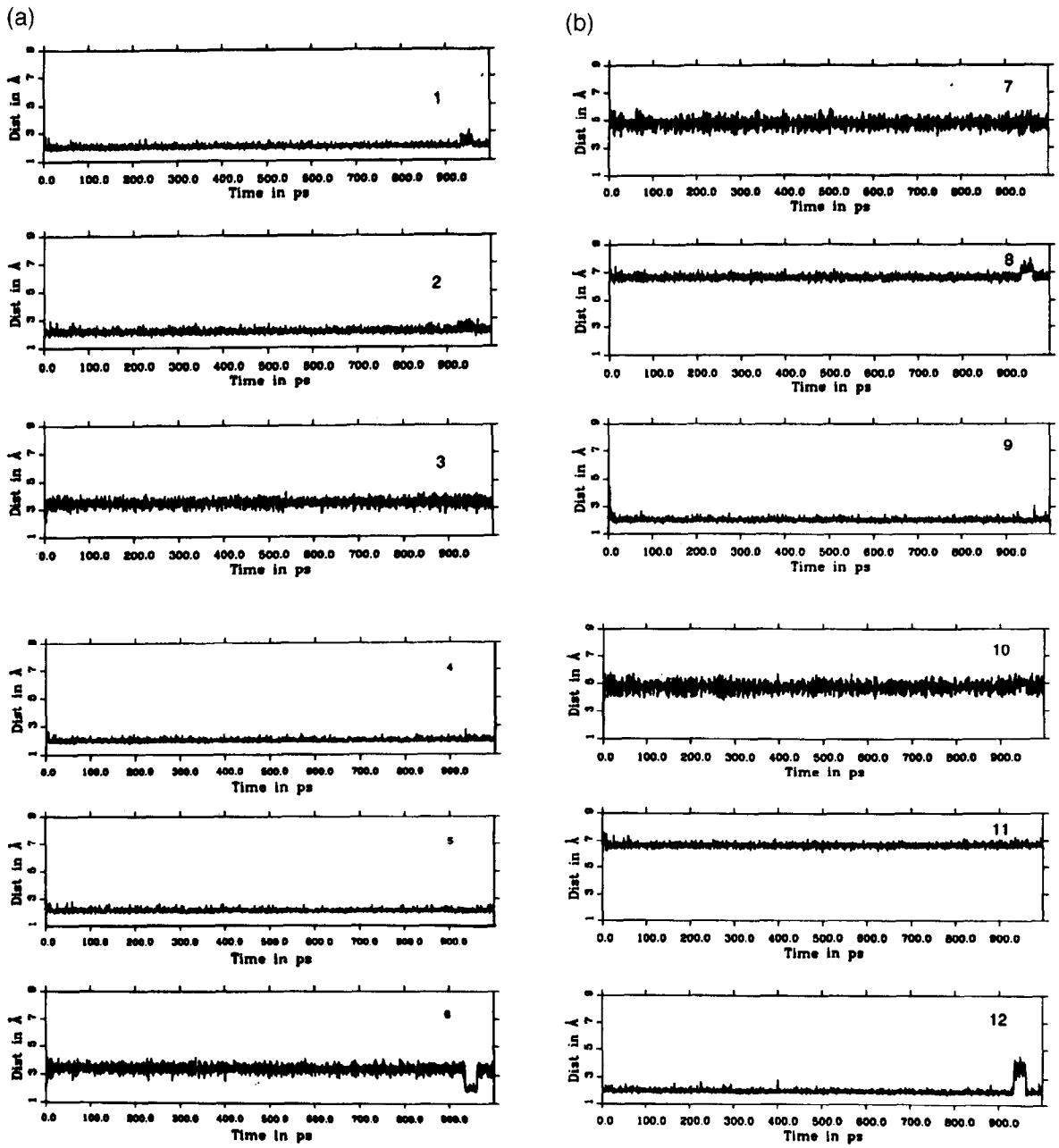


Fig. 9. The MD trajectories of hydrogen bonding distances. (a) 4 → 1 type, (b) 5 → 1 type of the 75 K simulation with alanine analog of valinomycin as the initial conformation. The numbers printed as inset in the trajectories correspond to the hydrogen bond number as given in bold in Table 1.

analogs, may have pronounced influence on the behavior of the latter in biological membranes.

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