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Molecular Dynamics Simulations of the TSSPSAD Peptide Antigen in Free and Bound with CAMPATH-1H Fab Antibody States: The Importance of the β-Turn Conformation

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Abstract Humanized CAMPATH-1H antibody has been found to have biological applications through the recognition of the CD52 antigen. A peptide mimotope of the CD52 antigen with the sequence $T_1SSPSAD_7$ has been co-crystallized with the CAMPATH-1H antibody. A plethora of hydrogen bond interactions were found to mediate antigen recognition. An important feature of peptide's bound conformation was the type I β-turn found in the S_3PSA_6 peptide's fragment. Paradoxically, this fact has been underestimated from other researchers. In order to further investigate the importance of this structural feature and its significance in antibody/antigen binding we have performed molecular dynamics simulations in explicit water of the $T_1SSPSAD_7$ peptide in both antibody free and bound states. We have found that the turn structure has been perfectly retained in the bound state but it was eliminated in the free state. This fact implies that the turn structure of the peptide is unstable in aqueous environment and it is induced upon antibody binding. Analysis of the trajectories revealed also several other important features of the antibody/antigen binding mode.

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Introduction

Antibodies are well-known multi-domain protein molecules for their capability of specifically bind other protein or non-protein molecules, the so called antigens (Sundberg and Mariuzza 2002). Functionality of antigen binding is localized close to N-terminal part of these chains which have significant variability in residue composition from antibody to antibody, V_H of the heavy chain and V_L of the light chain, respectively. Antigen binding topography (MacCallum et al. 1996) has been attributed to six fragments that constitute the Complementary Determining Regions (CDRs). Today there is a vast number of clinical (Jain et al. 2007; Nagorsen and Thiel 2007) or therapeutic (Shi et al. 2007; Liu et al. 2008) applications of antibodies.

CAMPATH-1H is a humanized (Almagro and Fransson 2008) monoclonal antibody against the CD52 antigen (Domagala and Kurpisz 2001). It has been used successfully for the treatment of leukemia, autoimmune disease and transplant rejection (Schmouder 2000; Dumont 2002). The CAMPATH-1H antibody has a highly basic binding site. It has been shown that the antibody binds the peptide mimotope $T_1SSPSAD_7$, and the complex structure has been determined by X-ray (James et al. 1999). The peptide's conformation in bound state was found to be type I β-turn around Pro_5Ser_6 residues. In contrast to other cases, where the CDR-H3 is essential to antigen binding (MacCallum et al. 1996), the CDR-L3 of CAMPATH-1H dominates antigen binding (James et al. 1999).

We present here a molecular dynamics (MD) study of the peptide antigen $T_1SSPSAD_7$ in both antibody free and bound states. This computational based approach has been shown to be very valuable and its importance is well described (Karplus and McCammon 2002; van Gunsteren et al. 2008). Similar computational approaches have been showed to add valuable information to existing experimental data of antibody/antigen complexes (Voordijk et al. 2000; Lorenzo et al. 2007), to identify "hot spots" in protein/protein recognition, or in searching for active conformational templates (Stavrakoudis et al. 2003; Tatsis et al. 2008). The dynamics of the turn structure of the peptide antigen, found in the crystal structure of the complex, is explored in bound state and its significance on the antibody binding is described. The work also provides a background for feature development of β-turn based therapeutics (Kee and Jois 2003).

Computational Methods

Initial coordinates were extracted from CAMPATH-1H complexed structure as deposited at PDB, access code 1ce1 (James et al. 1999). Starting conformation was built with the VMD program (Humphrey et al. 1996). Topology and force field parameters for all atoms were assigned from the CHARMM27 parameter set (MacKerell et al. 1998). The antibody/antigen complex was solvated with TIP3P (Jorgensen et al. 1983) water molecules using a rectangular box with dimensions $9.96 \times 8.49 \times 11.41$ nm^3. This allowed a distance of at least 1.8 nm between any protein atom and the edges of the box in order to avoid simulation artifacts (Weber et al. 2000). Water molecules found in the X-ray structure were also included and treated with the TIP3P model. The system was neutralized by placing 62 Na^+ and 70 Cl^- ions using VMD's autoionize plugin. From this point on, all subsequently MM and MD runs were performed with NAMD (v2.6) (Phillips et al. 2005) using 8 CPUs of a Linux cluster. Non-bonded van der Waals interactions were gradually turned off at a distance between 1.0 and 1.2 nm. The nonbonded pair list was updated every 10 steps at a distance 1.4 nm. Long range electrostatics were computed every second step with the PME method (Darden et al. 1993), with a grid spacing of less than 0.1 nm. Bonds to hydrogen atoms were constrained with the SHAKE (Ryckaert et al. 1977) with a relative tolerance of 10^{-8}, allowing a 2 fs step during MD runs. The whole system, consisted by 91076 atoms, was energy minimized with 5000 steps of conjugate gradients. After minimization the temperature of the system was gradually increased with Langevin dynamics, using the NVT ensemble, to 310 K, during a period 3000 steps, by stepwise reassignment of velocities every 500 steps. The simulation continued until 100,000 steps (0.2 ns). At minimization and equilibration stages, heavy atoms of the antibody/antigen complex (included the crystallographic water molecules) were restrained to their initial positions with a force constant of 50 kcal mol^{-1} Å^{-2}. The force constant of positional restraints was then decreased to 5 kcal mol^{-1} Å^{-2} for another 100,000 steps and finally positional restraints were totally eliminated for subsequent 200,000 steps of NVT equilibration period. The simulation was continued under constant pressure, with Langevin piston method (Feller et al. 1995), thus NPT ensemble, for 10 ns. Pressure was maintained at 1 atm and temperature was kept at 310 K. The results presented here are from this, isothermal–isobaric ensemble, MD run. Snapshots were saved to disk at 0.5 ps interval for further analysis. A similar trajectory was produced with only the peptide antigen present. The same computational procedure was applied. The length of this trajectory (free state) was 50 ns. Conformation analysis and visual inspection of structures were performed with VMD (Humphrey et al. 1996) and Carma (Glykos 2006) software packages, along with some in-house C++ code. Hydrogen bonds of the crystal structure were computed with the program HBPLUS (Mcdonald and Thornton 1994). Trajectory analysis of hydrogen bonds were performed with a single geometrical criterion: a hydrogen bond was accepted if the Acceptor-Hydrogen distance was <0.26 nm and the Donor-Hydrogen-Acceptor angle >120°. Secondary structure assignment was performed with STRIDE (Frishman and Argos 1995). Structural figures were prepared with PYMOL (Delano 2002). For the interpretation of colors in the figures the reader is referred to the online version of this article.

The root mean square deviation (RMSD) between the backbone atoms of the trajectory frames of polypeptide chains and the corresponding atoms of the X-ray structure, calculated for frame t, is given by Eq. 1, where x^m, y^m, z^m are the Cartesian coordinates found at the X-ray structure and x^t, y^t, z^t are the Cartesian coordinates of trajectory frame t. N is the number of atoms.

$$\mathrm{RMSD}_t = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i^m - x_i^t)^2 + (y_i^m - y_i^t)^2 + (z_i^m - z_i^t)^2} \quad (1)$$

The root mean square fluctuation (RMSF) of an atom is a measure of the deviation between the position of the atom and some reference position:

$$\mathrm{RMSF} = \sqrt{\frac{1}{T}\sum_{i=1}^{T}(x_i - \bar{x})^2} \quad (2)$$

where T is the number of trajectory frames and $\bar{x}$ is the time-averaged position. Practically, RMSF calculates the mobility of an atom during the MD trajectory, thus higher

RMSF values indicate higher mobility and lower RMSF values indicate restricted mobility.

Results and Discussion

Crystal Structure of the Antigen–Antibody Complex

The central part of the $T_1SSPSAD_7$ peptide was found in β-turn conformation in the crystal structure complexed with the CAMPATH-1H antibody (Fig. 1b). Secondary structure analysis with the STRIDE revealed that the peptide was in CCTTTTC conformational state (where C stands for Coil and T for Turn conformational states). Two consecutive β-turns were found. The fragment Ser_3–Pro–Ser–Ala_6 constituted the first type I β-turn which stabilized by the Ser_3:O–Ala_6:N hydrogen bond. The corresponding donor–acceptor distance was found 0.32 nm. Backbone bone dihedral angles (ϕ, ψ) were computed ($-73.1°$, $-15.0°$) and ($-93.0°$, $-1.5°$) for residues Pro_4 and Ser_5, respectively. These values are very close to those corresponding to an idealized type I β-turn, ($-60°$, $-30°$) and ($-90°$, $0°$) for residues $i+1$ and $i+2$, respectively. The fragment Pro_4–Ser–Ala–Asp_7 constituted the second type IV β-turn, which was not accompanied by hydrogen bond stabilization. The distance between Pro_4:C_α and Asp_7:C^α atoms was found 0.653 nm and the torsion angle Pro_4:C^α–Ser_5:C^α–Ala_6:C^α–Asp_7:C^α was found 81.3°. These data indicate that this turn is relatively unstable.

Another important intermolecular contact that stabilized the turn structure was the close contact between the side chain atoms Thr_1:C^γ and Ala_6:C^β. The corresponding distance was 0.33 nm (Fig. 1a), thus a relatively strong hydrophobic interaction was in presence.

Most of the antigen–antibody contacts involved antibody's light chain, especially the L3 region of CDRs. This was an unusual finding, since most of these contacts have been observed to involve the CDR-H3 (MacCallum et al. 1996). Numerous hydrogen bond interactions were also observed between the peptide antigen and antibody's CDRs, as it is shown at Fig. 5 of (James et al. 1999). This is well expected since the peptide contains numerous polar side chains. Table 1 lists the hydrogen bond interactions between the peptide antigen and antibody's light and heavy chains.

RMSF and RMSD Analysis

Figure 2 shows the RMSF of C^α atoms as calculated from the free and bound trajectories for the $T_1SSPSAD_7$ peptide. As it is expected, the peptide showed only limited mobility in the bound state and remarkable small RMSF values (<0.1 nm) confirm this fact.

Most of antibody's heavy and light chains C^α atoms showed RMSF values close to 0.1 nm. It is interesting to note that some exposed loop regions of the heavy chain showed increased RMSF values between 0.3 and 0.4 nm. For example, the RMSF peak at 0.4 nm corresponds to residue 165 of the heavy chain. Residues of the CDRs which were found to have contacts with the peptide antigen showed minimal RMS fluctuations between 0.05 and 0.1 nm (Fig. 2a). This fact indicates the relative rigidity of the CDRs.

RMSF values of peptide's C^α atoms of the MD trajectory of peptide in bound state were found between 0.06 and 0.1 nm (Fig. 2b). These low RMSF values underline the conformational rigidity of the peptide in the bound state. In the free state, RMSF values of peptide's C^α atoms were

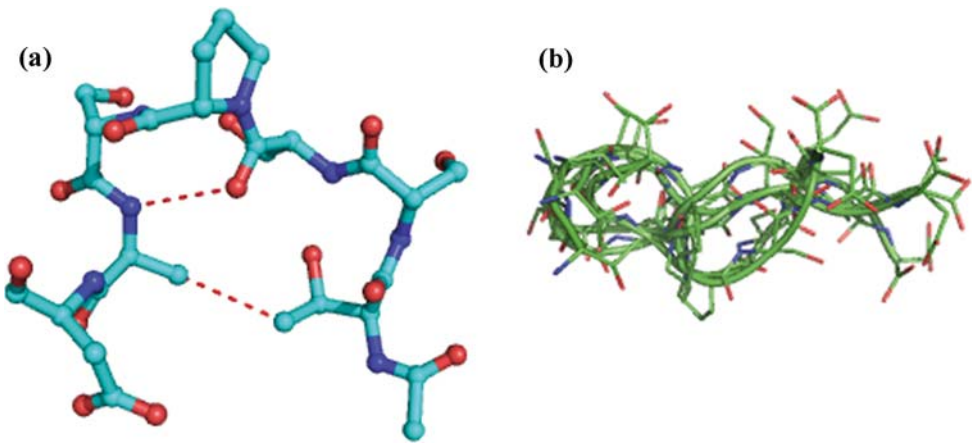

Fig. 1 **a** The TSSPSAD peptide in bound conformation as found in the X-ray structure of the antigen/antibody complex. Distances Ser_3:O–Asp_4:N (0.32 nm) and Thr_1:$C^{\gamma 2}$–Ala_6:C^β (0.33 nm) are highlighted, **b** 10 representative structures of the peptide during the MD trajectory in the free state, taken every 5 ns, demonstrating the loss of the initial β-turn conformation. Each frame is superimposed to the initial X-ray structure

Table 1 Hydrogen bonds found between the peptide antigen (P) and antibody's heavy (H) and light (L) chains. Percentage of frames (out of total number of trajectory frames) is given

Donor	Acceptor	Distance[a] (nm)	% Occurance
$Thr_{P1}:O^{\gamma}$	$Glu_{H101}:O^{\varepsilon 2}$	0.274	99
$Ser_{P2}:O^{\gamma}$	$Thr_{H104}:O$	0.261	98
$Ser_{P2}:N$	$His_{H103}:O$	0.300	27
$Ser_{P3}:O^{\gamma}$	$His_{L91}:N^{\delta 1}$	0.263	99
$Ser_{P5}:O^{\gamma}$	$His_{L91}:O$	0.263	92
$Ser_{P5}:N$	$His_{L91}:O$	0.263	26
$Arg_{L94}:N^{\varepsilon}$	$Ser_{P5}:O$	0.300	34
$Arg_{L96}:N^{\varepsilon}$	$Ser_{P5}:O^{\gamma}$	0.284	99
$Arg_{L94}:N^{\eta 1}$	$Asp_{P7}:O^{\delta 1}$	0.584	19
$Arg_{L94}:N^{\eta 1}$	$Asp_{P7}:O^{\delta 2}$	0.738	39
$Tyr_{H33}:O^{\eta}$	$Asp_{P7}:O^{\tau 1}$	0.724	9
$Tyr_{H33}:O^{\eta}$	$Asp_{P7}:O^{\tau 2}$	–	70
$Tyr_{H33}:O^{\eta}$	$Asp_{P7}:O^{\delta 1}$	0.272	13
$Tyr_{H33}:O^{\eta}$	$Asp_{P7}:O^{\delta 2}$	0.238	8
$Arg_{H52}:N^{\varepsilon}$	$Asp_{P7}:O^{\delta 1}$	0.244	20
$Arg_{H52}:N^{\varepsilon}$	$Asp_{P7}:O^{\delta 2}$	0.447	93
$Arg_{H52}:N^{\eta 2}$	$Asp_{P7}:O^{\delta 1}$	0.348	20
$Arg_{H52}:N^{\eta 2}$	$Asp_{P7}:O^{\delta 2}$	0.564	93

[a] Donor–acceptor distance found in the crystal structure

found between 0.15 and 0.28 nm. These significantly increased values indicate the higher (as expected) flexibility of the peptide in the solution. Higher RMSF values were recorded for residues close to N- and C-terminals, especially for the Asp_7 residue (0.28 nm), located at the C-terminal part of the peptide. On the other hand, the Ser_3 residue showed the smaller RMSF value in both MD trajectories, with the peptide in free and bound state (0.063 and .14 nm, respectively). In general, RMSF values of peptide's C^{α} atoms were found with remarkable covariance in free and bound states. It is assumed that the mobility of the peptide was governed by intrinsic factors and that the antibody suppressed down this mobility.

Antibody's heavy and light chains showed moderate mobility during the MD trajectory, as revealed from backbone RMSD time series plot (Fig. 3a). RMSD of the heavy chain exhibited values between 0.13 and 0.34 nm which averaged at 0.23 nm (0.04 nm). Light chain showed less mobility than the heavy one with RMSD values between 0.10 and 0.26 nm which averaged at 0.17 nm (0.03 nm). These time series were relative stable through the simulation time (especially after the 1st ns) which indicates the stability of the trajectory.

Peptide's backbone atoms RMSD time evolution (in the bound state, Fig. 3a) showed very limited fluctuation and

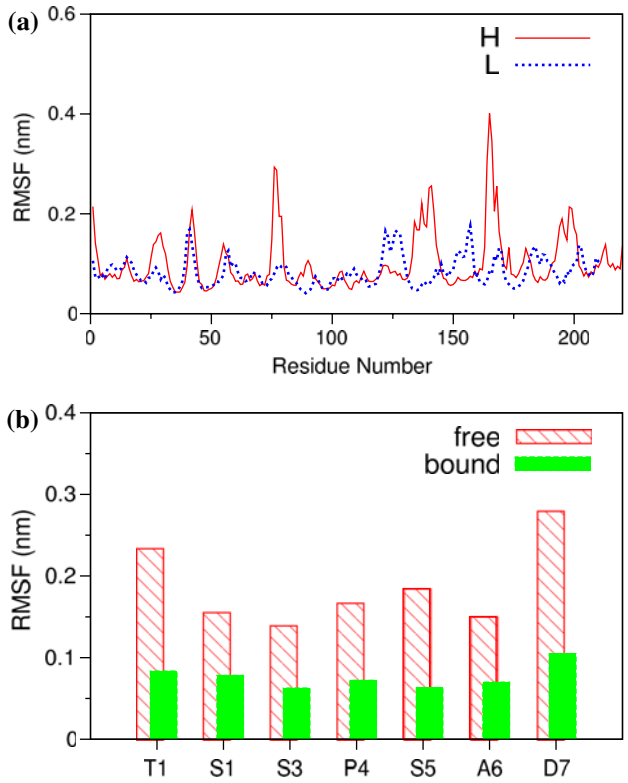

Fig. 2 RMSF of the antibody's (**a**) and the peptide's (**b**) C^{α} atoms (in both free and bound states). Antibody's heavy (H) and light (L) chains of part (**a**) are colored red and blue, respectively

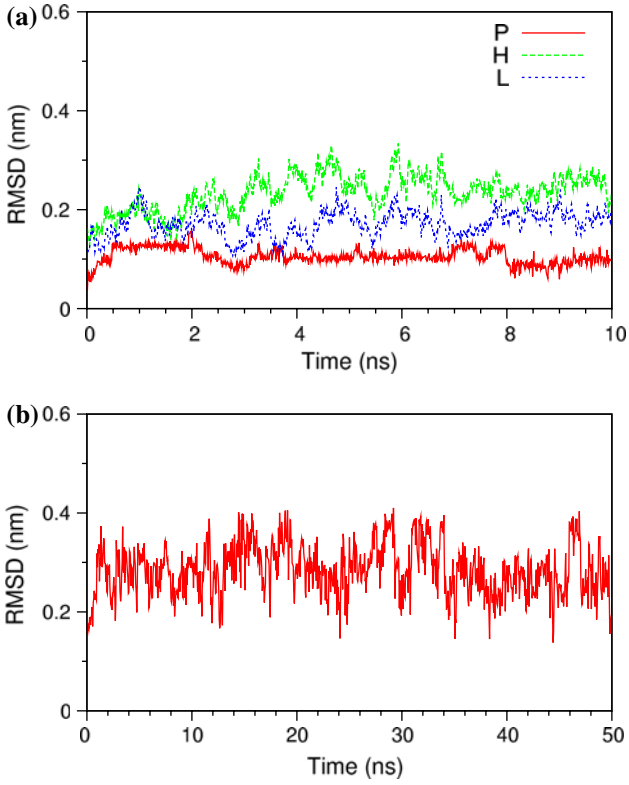

Fig. 3 RMSD time evolution of backbone atoms of the peptide in bound (**a**) and free (**b**) states. H and L stand for antibody's heavy and light chains, respectively, P stands for the peptide antigen

recorded values were found to be between 0.04 and 0.16 nm. Time series average value was found 0.11 nm (0.016 nm). These relatively low values, along with the previously analyzed RMS fluctuations, indicate the very low flexibility of the peptide when bound to antibody's binding site. A trajectory movie (supplement material) also provides visual evidence about this fact. The CAMPATH-1H antibody engulfed the peptide antigen and no part or fragment of it was allowed to move. These observations support other structural studies (James et al. 1999) about the affinity of the CAMPATH-1H antibody for this minimal peptide mimotope of the CD52 antigen.

Contrary to the previous observations concerning the RMSD of the peptide bound to the antibody, when the peptide was simulated in the absence of the antibody (free state) it showed significantly increased mobility (Fig. 3b). Backbone RMSD values of the peptide ranged between approximately 0.1 and 0.42 nm during the 50 ns trajectory. These values averaged at 0.28 nm (0.05 nm). RMSD fluctuation remained relative stable during MD trajectory and the time series of RMSD was found to be homeskedastic (Fig. 3b). In line with the RMSF analysis, RMSD analysis indicates that the peptide did not retained its initial conformation.

Backbone Conformation and Secondary Structure of the Peptide Antigen

The initial conformational state of the $T_1SSPSAD_7$ was CCTTTTC according to STRIDE assignment. Trajectory analysis of the peptide's secondary structure with STRIDE was revealed that this conformation was retained for 99.6% of the frames. This fact, along with the very low backbone RMSD and C^{α} RMSF values analyzed previously, indicates that the peptide's conformation was almost freezed into the binding site of the CAMPATH-1H antibody.

The type I β-turn structure observed at the Ser_3–Pro–Ser–Ala_6 peptide's fragment was very well conserved. Time series and Ramachandran plots of backbone dihedral angles ϕ, ψ of the residues Pro_4 and Ser_5, lying at the middle of the β-turn fragment are shown at Fig. 4. The minimal fluctuations of these dihedrals around the initial values are clearly demonstrated from these graphs.

Backbone dihedrals ϕ, ψ of Pro_4 averaged at $-77.0°$ and 5.6°, respectively, very close to the values found at the crystal structure ($-73.1°$, $-15.0°$). Similarly, time series of backbone dihedrals ϕ, ψ of Ser_5 showed remarkable stability and averaged at $-73.1°$ and $-15.0°$, respectively, which they are also very close to the initial values ($-93.0°$, $-1.5°$). The peptide's conformation showed very limited mobility during the MD trajectory of the peptide in the bound state (Fig. 4a) and the values of these dihedral angles did not changed significantly. Moreover, the main structural feature of the peptide antigen, thus the β-turn of the fragment Ser_3–Pro–Ser–Ala_6 was perfectly conserved during the MD trajectory. It is assumed that this is a prerequisite for the binding affinity and it should be taken into consideration for future development. Statistical analysis of backbone dihedrals (ϕ_4, ψ_4, ϕ_5, ψ_5) revealed that 32.7% of the trajectory frames had all of these four dihedral values within $\pm 15°$ of the initial values. This percentage was increased to 90.6% when a 30° cutoff criterion was applied. When the dihedral values were compared with the values that correspond to idealized type I β-turn: ($-60°$, $-30°$) and ($-90°$, 0°), the above percentages where found somewhat decreased. Thus, 6.3% of the trajectory was found within $\pm 15°$ cutoff and 63.9% when the 30° cutoff was applied.

Figure 5 shows the time evolution of the Ser_3:O–Ala_6:H^N distance, which corresponds to the β-turn. For the peptide in the bound state, this distance value fluctuated between 0.17 and 0.49 nm and averaged at 0.256 nm (0.033 nm) (Fig. 5a). For 45.3% of the trajectory frames Ser_3:O–Ala_6:H^N distance was found <0.25 nm, which is a general cutoff for hydrogen bonds. Although, the existence of a hydrogen bond is not an absolute requirement for the acceptance of a β-turn structure, it is clear that its presence stabilized the β-turn. The turn structure of the Ser_3–Pro–Ser–Ala_6 was also confirmed by Ser_3:C^{α}–Ala_6:C^{α} distance (Fig. 5c) which fluctuated in the range (0.51 nm, 0.72 nm) and averaged at 0.59 nm (0.03). The corresponding dihedral angle (four C^{α} atoms of the sequence) varied between 51.6° and 97.3° and averaged at 73.2°. It is generally accepted that distances of <0.7 nm and dihedral angle (absolute) values of less then 90° are accompanied with β-turn structure. Overall, time series analysis of backbone dihedral angles, backbone hydrogen bond and C^{α} distance advocate to the existence of type I β-turn structure of the Ser_3–Pro–Ser–Ala_6 fragment.

In contrast, simulation of the peptide in the free state showed that the initial turn conformation was lost during the MD trajectory (Fig. 5b, d). STRIDE assigned 86.2% of the recorded frames to be in CCCCCCC conformation, thus an unordered structure was obtained. The initial CCTTTTC conformational state was found in only 4.8% of the frames during the 50 ns trajectory. These frames were distributed almost uniformly through the trajectory. This indicates that although the initial conformation was lost, it remained accessible for the peptide in aqueous solution.

The disappearance of the turn structure is clearly evident from Fig. 4b, where the time series of backbone dihedral angle are plotted. Conformational transitions were more evident for the ψ dihedral angle. Only 0.1% of the trajectory frames had backbone dihedral angles (ϕ_4, ψ_4, ϕ_5, ψ_5) with 30° of the initial values. Moreover, time series of the Ser3:O–Ala6:H^N distance (Fig. 5b) that corresponds to the

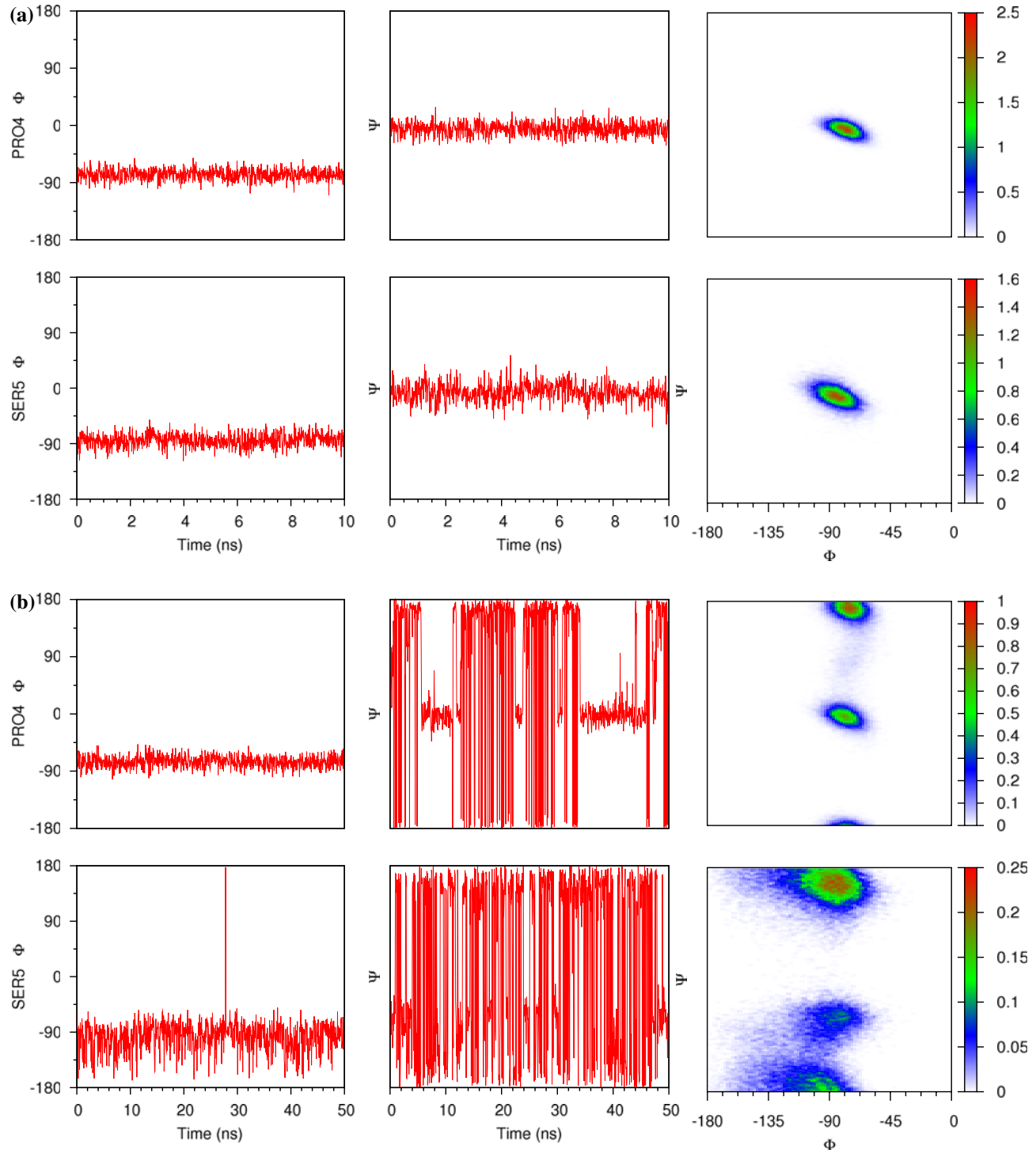

Fig. 4 Backbone dihedrals (ϕ on the left, ψ on the middle, Ramachadran map on the right) for the residues Pro_4 and Ser_5 of the peptide from the bound (**a**) and free (**b**) trajectories. Two dimension probability density plot obtained for the residues Pro_4 and Ser_5 of the peptide from the bound (a) and free (b) trajectories. The adjacent color bar is used to identify regions of low (blue) versus high (red) population

hydrogen bond stabilizing the β-turn fluctuated between 0.18 and 0.87 nm and averaged at 0.66 nm (0.09 nm), which means that the hydrogen bond interaction was lost. A similar conclusion can be drawn from the time series analysis of the Ser_3:C^{α}–Ala_6:C^{α} distance (Fig. 5d) which was found to be greater from 0.7 nm for the majority of

trajectory frames. This distance fluctuated between 0.48 and 1.10 nm with average value of 0.86 nm (0.13 nm).

More evidence for the disappearance of the folded β-turn structure has been provided from the analysis of the distance between C^{α} atoms of residues Thr_1 and Asp_7 (end-to-end distance). Time series of this distance (Fig. 6b) corresponding to the peptide at bound state averaged at 0.80 nm (0.09 nm) minimum and maximum values 0.5 and 1.0 nm, respectively. On the contrary, significantly increased values were observed during the trajectory of the peptide in the free state where the end-to-end distance averaged at 1.5 nm (0.3 nm) and fluctuated between 0.5 and 2.1 nm. The end-to-end distance for a 7mer peptide modeled at extended conformation ($\phi = -135^{\circ}$, $\psi = 135^{\circ}$) was found 2.1 nm. This finding indicates that the peptide spent most of the simulation time in a structure close to fully extended conformation.

Hydrophobic Interactions

An important hydrophobic contact between Thr_1:C^{γ} and Ala_6:C^{β} atoms was traced in the peptide's conformation found in the X-ray structure of the antigen/antibody complex. The corresponding distance was found 0.332 nm. This is a remarkable close contact, which remained intact during the MD trajectory of the peptide in the bound state. Time series of the Thr_1:C^{γ}–Ala_6:C^{β} distance during MD trajectory of the bound state (Fig. 6a) fluctuated between

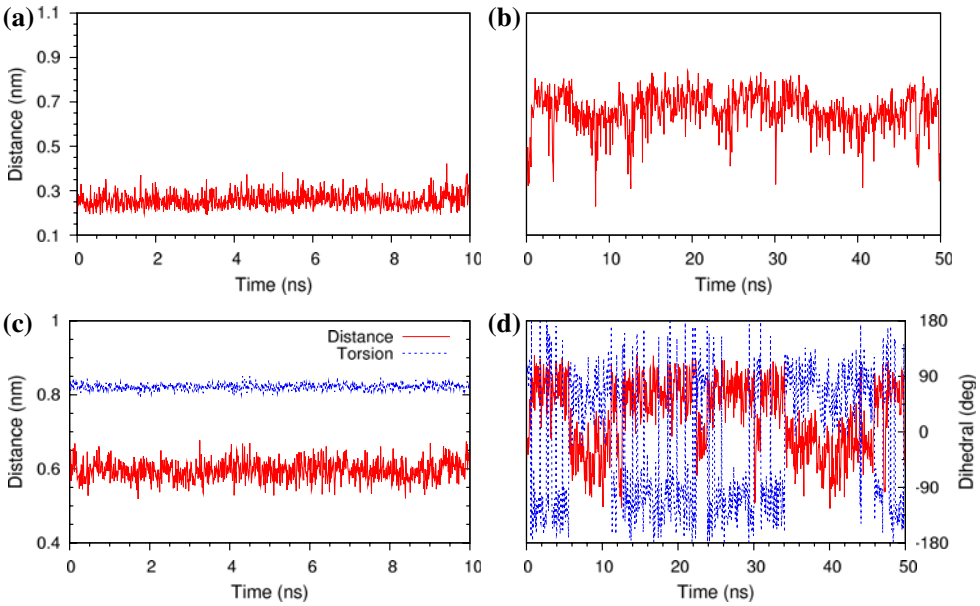

Fig. 5 **a** Time series of the Ser_3:O–Asp_4:H^N distance from the MD trajectory of the peptide in the bound state, **b** time series of the Ser_3:O–Asp_4:H^N distance from the MD trajectory of the peptide in the free state, **c** Ser_3:C^{α}–Ala_6:C^{α} distance (red line) and Ser_3:C^{α}–Pro_4:C^{α}–Ala_5:C^{α}–Ala_6:C^{α} dihedral angle (blue line) time evolution from the MD trajectory of the peptide in the bound state and **d** Ser_3:C^{α}–Ala_6:C^{α} distance (red line) and Ser_3:C^{α}–Pro_4:C^{α}–Ala_5:C^{α}–Ala_6:C^{α} dihedral angle (blue line) time evolution from the MD trajectory of the peptide in the free state

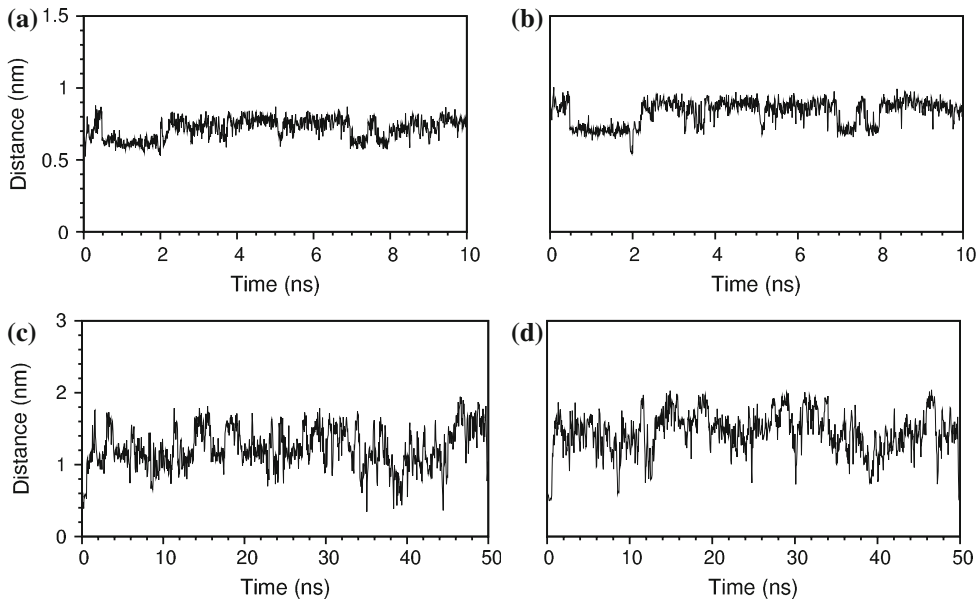

Fig. 6 Time series of some important distances of peptide atoms: **a** Thr_1:$C^{\gamma 2}$–Ala_6:C^{β} distance from the MD trajectory of the peptide in the bound state, **b** Thr_1:C^{α}–Asp_7:C^{α} distance from the MD trajectory of the peptide in the bound state, **c** Thr_1:$C^{\gamma 2}$–Ala_6:C^{β} distance from the MD trajectory of the peptide in the free state and **d** Thr_1:C^{α}–Asp_7:C^{α} distance from the MD trajectory of the peptide in the free state

0.31 and 0.57 nm and averaged at 0.39 nm (0.03 nm). Under the assumption that a hydrophobic contact occurs when the corresponding distance of heavy atoms is <0.55 nm, then these two atoms was in hydrophobic contact in 99.96% of the trajectory frames. On the contrary, this interaction was almost in absence during the MD trajectory of the peptide in the free state (Fig. 6c). The corresponding distance averaged at 1.22 nm (0.29 nm) with minimum and maximum values 0.34 nm and 1.99 nm, respectively.

Antibody–Antigen Interactions

The crystal structure of the antibody/antigen complex revealed numerous hydrogen bond interactions (James et al. 1999). The majority of them involved the CDR-L3 region of the antibody. Table 1 lists these hydrogen bonds along with the percentage of appearance during the MD trajectory of the peptide in the bound state after the corresponding time series analysis. The majority of these interactions were found in presence during the MD trajectory of the peptide in the bound state but some hydrogen bond interactions were relatively weak under MD conditions (Table 1).

Five hydrogen bonds, Thr_{P1}:O^{γ}–Glu_{H101}:$O^{\varepsilon 2}$, Ser_{P2}:O^{γ}–Thr_{H104}:O, Ser_{P3}:O^{γ}–His_{L91}:$N^{\delta 1}$, Ser_{P5}:O^{γ}–His_{L91}:O and Arg_{L96}:N^{ε}–Ser_{P5}:O^{γ}, that existed in antibody/antigen complex were very well conserved during the MD trajectory. More than 90% of the frames satisfied the geometrical criteria for hydrogen bond acceptance (Table 1). Two other hydrogen bonds, Ser_{P5}:N–His_{L91}:O and Arg_{L94}:N^{ε}–Ser_{P5}:O found in the crystal structure, were preserved less well during MD trajectory. Geometrical analysis revealed that these hydrogen bonds were in presence for 26% and 34% of the frames, respectively. It is interesting to note that breaking of the Arg_{L94}:N^{ε}–Ser_{P5}:O hydrogen bond was accompanied by formation of the Arg_{L94}:$N^{\eta 1}$–Asp_{P7}:$O^{\delta 2}$ hydrogen bond for 39% of the frames. The corresponding distance was found 0.738 nm in the crystal structure. This means that this hydrogen bond was not in presence in the starting structure and it was formed during the MD trajectory.

Rearrangement of the chain interactions was also observed between peptide's Asp_7 and antibody's Arg_{H52} and Tyr_{H33}. The initial hydrogen bonds Tyr_{H33}:O^{η}–Asp_{P7}:$O^{\delta 1}$, Tyr_{H33}:O^{η}–Asp_{P7}:$O^{\delta 2}$ and Arg_{H52}:N^{ε}–Asp_{P7}:$O^{\delta 1}$ were poorly conserved during the MD trajectory. New hydrogen bonds were established in their place: Tyr_{H33}:O^{η}–Asp_{P7}:$O^{\tau 2}$, Arg_{H52}:N^{ε}–Asp_{P7}:$O^{\delta 2}$ and Arg_{H52}:$N^{\eta 2}$–Asp_{P7}:$O^{\delta 2}$.

It has been shown that a negatively charged residue at the C-terminal of the peptide (Asp_7) is essential for antibody binding and the importance of charge complementarity has been strongly argued. Molecular dynamics simulation presented here strongly supports this fact, in line with recently published similar studies (Sinha et al. 2007). The side chain carboxylic group of the Asp_7 residue was found to participate in hydrogen bonds or salt bridges interactions with the positively charged pocket of the antibody binding site (Table 1).

Another important interchain interaction that contributed significantly to the antibody binding of the antigen was between peptide's Pro_4 and antibody light chain Tyr_{32} side chains. For example, distance between Pro_{P4}:C^{γ}–Tyr_{L32}:C^{γ} atoms fluctuated between 0.33 and 0.57 nm and averaged at 0.42 nm (0.03 nm).

Given the plethora and stability of the antidody/peptide interactions, the "freezed" conformation of the peptide in the bound state during the MD trajectory looks now obvious. Results presented previously about the very low RMSF values of the peptide's C^{α} atoms in the bound state, conforms with the tight binding of the peptide through hydrogen bonds and hydrophobic interactions that took place.

Concluding Remarks

Molecular dynamics simulations have been utilized to explore the conformational properties of a peptide antigen in both free and bound state to CAPMATH-1H antibody. The importance of the electrostatic interactions between a negatively charged residue at C-terminal of peptide antigen and the positively charged pocket of antibody's binding site has been showed here to be very important. Although side chain of aspartic residue dominated these interactions, α-carboxylic group was found also to be important.

One important result of the current study, somewhat underestimated from previous structural studies, is the existence of a type I β-turn found in the Ser_3–Pro–Ser–Ala_6 fragment of the bound peptide. Turn conformations are well-known motifs of protein–protein interactions and recognition processes. This turn was very well conserved during the MD trajectory of the peptide in the bound state but it was almost in total absence in the MD trajectory of the peptide in the free state. This result suggests that the peptide does not adopt a well-organized structure in aqueous environment (Fig. 1) and the antibody imposed this turn conformation during the binding process. Stabilization of this turn structure, through peptidomimetics or cyclization for example, could possibly enhance the binding affinity of the peptide and could lead to new more efficient bioactive compounds.

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