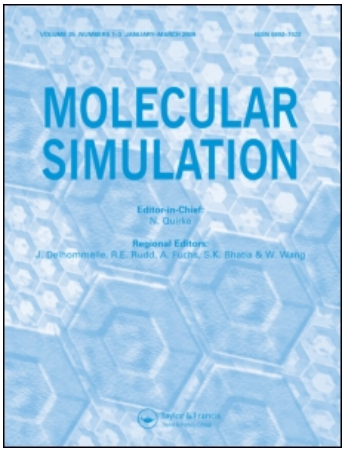

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Computational modelling and molecular dynamics simulations of a cyclic peptide mimotope of the CD52 antigen complexed with CAMPATH-1H antibody

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Computational modelling and molecular dynamics simulations of a cyclic peptide mimotope of the CD52 antigen complexed with CAMPATH-1H antibody

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A peptide mimotope of the CD52 antigen with the sequence $T_1SSPSAD_7$ has been co-crystallised with the CAMPATH-1H antibody. Molecular dynamics (MD) simulations in explicit water of the $T_1SSPSAD_7$ peptide in both antibody free and bound states showed that the peptide's β-turn remained stable in the bound state but it was eliminated in the free state. Based on the observation that Thr_1 and Ala_6 residues made close contacts through their side chain, a new peptide mimotope is proposed: (S,S)-$C_1SSPSCD_7$. Thr_1 and Ala_6 residues have been mutated in Cys residues and a disulphide bond has been imposed. The new analogue has been simulated in both antibody bound and free states with MD in explicit water. It was found that the peptide remained in the stable β-turn conformation, both in complexed and free states. The difference in configurational entropy was estimated to be 0.15 kJ/K/mol. However, despite the structural similarity, the cyclic analogue lost more than 25% of its buried surface area contact with the antibody and a couple of critical hydrogen bond interactions were broken. It is concluded that design of cyclic analogues that mimic the bound conformation of peptides should be carefully performed and conformational 'freezing' does not necessarily guarantee better binding.

Keywords: antigen/antibody interactions; CAMPATH-1H; CD52 antigen; computer simulation; disulphide cyclisation; molecular dynamics; QSAR; β-turn

1. Introduction

Antibodies are multi-domain protein molecules well known for their capability of specifically binding other protein or non-protein molecules, the so-called antigens [1]. Functionality of antigen binding is localised close to N-terminal part of their protein chains that have significant variability in residue composition from one antibody to another, V_H of the heavy chain and V_L of the light chain, respectively. Antigen binding topography [2] has been attributed to six fragments that constitute the complementary determining regions (CDRs). Today there are a vast number of clinical [3,4] or therapeutic [5,6] applications of antibodies.

CAMPATH-1H is a humanised [7] monoclonal antibody against the CD52 antigen [8]. It has been used successfully for the treatment of leukaemia, autoimmune disease and transplant rejection [9,10]. 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 [11]. 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 [2], the CDR-L3 of CAMPATH-1H dominates antigen binding [11]. Molecular dynamics (MD) simulations of the $T_1SSPSAD_7$ peptide in complex (with CAMPATH-1H) and free states revealed that β-turn conformation of the peptide is retained only in the complexed state [12]. This fact indicates that the antibody induces the β-turn conformation upon complexation process.

The design of therapeutic compounds based on β-turn conformation is a well known process [13]. It is possible that stabilisation of the β-turn in the Pro_5Ser_6 fragment could enhance binding properties of the peptide mimotope. Among the many ways to introduce structure stabilisation in a peptide fragment, one of the most frequently used is disulphide cyclisation [14–17]. Other notable examples of biological activity enhancement through peptide cyclisation can be found in the literature [18,19]. The $T_1SSPSAD_7$ peptide directly offers an opportunity. Here, a MD study of the peptide mimotope (S,S)$C_1SSPSCD_7$ of the CD52 antigen is presented, in both antibody free and bound states. This side chain proximity of Thr_1 and Ala_6 of the original mimotope offers an excellent opportunity for disulphide engineering: Thr_1 and Ala_6 can be substituted by cysteine residues and a cyclic structure can be imposed through disulphide bond formation. Since there are no major conformational changes to be expected, the resulted cyclic peptide can be used instead of its original linear precursor under the assumption that the antibody can bind the designed peptide.

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MD simulations offer a solid background for studying biomolecular structure and interactions. This computational-based approach has been shown to be very valuable and its importance is well described [20–23]. Similar computational approaches have been shown to add valuable information to existing experimental data of antibody/antigen complexes [24–26], in searching for active conformational templates [27,28], to establish a model of protein/peptide dimerisation based on a disulphide bridge [29], or to explore pathways of protein folding [30]. This study presents the engineering of a peptide mimotope through disulphide cyclisation and exploration of its structural properties in bound and free states through MD simulations. It is proposed that the cyclic peptide has stable β-turn conformation in solution and its structure at the bound state is very close to the crystal structure of its linear precursor bound at the CAMPATH-1H antibody's binding site. Reduction of the entropy required for binding of the $T_1SSPSAD_7$ peptide could possibly result in better binding properties. On the other hand, restriction on mobility can hinder small or even subtle conformational changes needed for optimal binding and biological activity. MD simulations offer an excellent framework to study both aspects of the proposed engineering of the linear peptide: (1) if the cyclic peptide retains in free and bound states the bound conformation of the linear analogue and (2) if the cyclic peptide retains good binding affinity as the linear analogue.

2. Methodology

2.1 Computational modelling of the system

Initial coordinates were extracted from CAMPATH-1H complexed structure as deposited at PDB, access code 1ce1 [11]. Molecular modelling of the disulphide cyclic $(S,S)C_1SSPSCD_7$ peptide and starting conformation for the subsequent molecular mechanics MM/MD studies were built with the VMD program [31]. Residues Thr_1 and Ala_6 of chain P (peptide) were mutated to Cys and a disulphide bond was imposed. Topology and force field parameters for all atoms were assigned from the CHARMM27 parameter set [32]. The antibody/antigen complex was solvated with TIP3P [33] water molecules using a rectangular box of 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 artefacts [34]. Water molecules found in the X-ray structure were also included and treated with the TIP3P model. The system was neutralised by placing 62 Na^+ and 70 Cl^- ions, using VMD's autoionise plugin, in order to approximate the ionic strength of 0.25 M. From this point on, all subsequent MM and MD runs were performed with NAMD (v2.6) [35] using eight 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 non-bonded pair list was updated every 10 steps at a distance of 1.4 nm. Long-range electrostatics were computed every second step with the PME method [36] with a grid spacing of less than 0.1 nm. Bonds to hydrogen atoms were constrained with the SHAKE [37] with a relative tolerance of 10^{-8}, allowing a 2 fs step during MD runs. The whole system, consisting of 91,076 atoms, was energy minimised with 5000 steps of conjugate gradients. After minimisation, the temperature of the system was gradually increased with Langevin dynamics, using the NVT ensemble, to 310 K, during a period of 3000 steps, by stepwise reassignment of velocities every 500 steps. The simulation continued until 100,000 steps (0.2 ns). At minimisation 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/Å^2. The force constant of positional restraints was then decreased to 5 kcal/mol/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 [38], thus NPT ensemble, for 20 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 1 ps intervals 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.

2.2 Conformational analysis

Conformational analysis and visual inspection of structures were performed with VMD [31], Carma [39] and Eucb [40] software packages. Appropriate circular statistics were taken into consideration for calculation of descriptive values of dihedral angles [41]. Trajectory analysis of hydrogen bonds was performed with a single geometrical criterion: a hydrogen bond was accepted if the acceptor–donor distance was less than 0.33 nm and the donor–hydrogen–acceptor angle greater than 120°. Hydrophobic interactions were assigned when two non-polar heavy atoms were in less than 0.4 nm distance proximity. Secondary structure assignment was performed with STRIDE [42]. Structural figures were prepared with PYMOL [43].

The root mean square distance (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 Equation (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.

$$\mathrm{RMSD}_t = \frac{1}{N}\sqrt{\sum_{i=1}^{N}\left(x_i^m - x_i^f\right)^2 + \left(y_i^m - y_i^f\right)^2 + \left(z_i^m - z_i^f\right)^2}. \tag{1}$$

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:

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

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. RMSD and RMSF calculations were performed after removing the global translation/rotation of the protein system by atom-positional least-squared fitting the protein atoms of the trajectory onto the coordinates of the first frame.

2.3 Buried surface calculation

Calculation of buried surface area (BSA) was performed with the NACCESS program [44], based on the formula:

$$\mathrm{BSA} = \mathrm{Sp} + \mathrm{Sa} - \mathrm{Sc}, \tag{3}$$

thus as the difference of the surface accessible area of the complex (Sc) from the sum of the surface accessible areas of the peptide (Sp) and antibody (Sp), respectively. Calculations were performed to all frames of the compP (peptide/antibody complex) trajectory in order to get and characterise the times series of BSA.

2.4 Entropy calculations

Schliter's formulation [45] was used for the calculation of the configurational entropy (S):

$$S_{\mathrm{true}} < S = \frac{1}{2}k_{\mathrm{B}} \ln \det\left[1 + \frac{k_{\mathrm{B}}Te^2}{\hbar^2}\boldsymbol{M}\boldsymbol{\sigma}\right], \tag{4}$$

where S is an upper estimation of the true entropy (S_{true}), k_{B} is Boltzmann's constant, T is the absolute temperature (in which the system was simulated), e is Euler's number, $\hbar$ is Plank's constant divided by 2π, M is the mass matrix that holds on the diagonal the masses belonging to the atomic Cartesian degrees of freedom and σ is the covariance matrix of atom positional fluctuations:

$$\boldsymbol{\sigma}_{ij} = (x_i - \langle x_i\rangle)(y_i - \langle y_i\rangle). \tag{5}$$

Entropy calculations were performed with the backbone atoms (N, C^{α} and C') of the peptide from the bound and free trajectories, respectively, at 0.1 ns interval (100 frames).

Two separate trajectories (for example free and complex trajectories of a peptide) can be combined, thus one trajectory can be appended at the end of the other trajectory, and the plot of configurational entropy S against time can be used as assessment of the overlap between configurational spaces sampled in two simulations [46]. Such trajectories have been derived for the backbone (bb) atoms (N, C^{α} and C') of the peptide from the last 10 ns of the free (f) and bound (b) trajectories. Both appending sequences were applied resulting in S_{bb}^{f+b} and S_{bb}^{b+f} calculations, where the bound trajectory was appended to the free ($f + b$) or the free trajectory was appended to the bound one ($b + f$). Plotting the calculated values of S from both the combined trajectories over time demonstrates the relative size and overlap of sampled trajectories. Plotting of S over time after the combination of two trajectories results in three cases [46], briefly described as:

(1) S increases after appending one trajectory after another, with a jump observed at this point, thus the two trajectories do not overlap, or there is only a small overlap between them.
(2) S evolves smoothly after the appending of the trajectories, without an observable perturbation of the line of S over time, thus the two trajectories show significant overlap.
(3) S curve increases during the time of the first trajectory but decreases a little after the appending of the second trajectory, thus the second trajectory samples a smaller configurational space than the first one, which also contains the configurational space visited by the second one.

Such an analysis of the combined trajectories is more advantageous than comparing directly the configurational entropies of two independent trajectories and can provide information about the extent of sampling overlap (if any) between two trajectories. The coverage of the free trajectory with respect to the complex trajectory is:

$$\Delta S_{\mathrm{bb}}^{b+f} \equiv S_{\mathrm{bb}}(\mathrm{complex, peptide}) - S_{\mathrm{bb}}(\mathrm{complex}), \tag{6}$$

and the coverage of the bound (complex) trajectory with respect to the free trajectory is:

$$\Delta S_{\mathrm{bb}}^{f+c} \equiv S_{\mathrm{bb}}(\mathrm{peptide, complex}) - S_{\mathrm{bb}}(\mathrm{peptide}). \tag{7}$$

3. Results and discussion

3.1 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. 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 stabilised by the Ser_3:O-Ala_6:N hydrogen bond. The corresponding donor–acceptor distance was found to be 0.32 nm. Backbone dihedral angles (ϕ,ψ) were (−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 idealised 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 stabilisation. The distance between Pro_4:C_α and Asp_7:C^α atoms was found to be 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 to be 81.3°. These data indicate that this turn is relatively unstable.

Another important intermolecular contact that stabilised 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, thus a relatively strong hydrophobic interaction was in presence.

Most of the antigen–antibody contacts involved the 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 [2]. Numerous hydrogen bond interactions were also observed between the peptide antigen and antibody's CDRs, as it is shown at Figure 5 of [11]. This is to be 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.

Table 1. Hydrogen bonds found between the peptide mimotope (P) and antibody's heavy (H) and light (L) chains.

Donor	Acceptor	Distance[a] (nm)	% Occurence
Ser_{P2}:O^γ	His_{H103}:O	0.300	–
Ser_{P2}:N	His_{H103}:O	0.300	32
His_{L91}:$N^{\delta1}$	Ser_{P3}:O^γ	0.263	28
Ser_{P3}:O^γ	His_{L91}:O	0.263	54
Arg_{L94}:$N^{\eta2}$	Pro_{P4}:O	0.680	57
Arg_{L94}:$N^{\eta1}$-Arg_{L96}:$N^{\eta6}$	Ser_{P5}:O	0.300	86
Arg_{L96}:$N^{\varepsilon,\eta2}$	Ser_{P5}:O^γ	0.284	87
Arg_{H52}:$N^{\eta2}$	Cys_{P6}:O	0.330	65
Ser_{P5}:O^γ	His_{L91}:O	0.263	63
Ser_{P5}:N	His_{L91}:O	0.263	–
Arg_{H52}:N^ε	Asp_{P7}:$O^{\delta1}$	0.244	–
Tyr_{H33}:O^η	Asp_{P7}:$O^{\delta1}$	0.272	3
Lys_{H56}:N^ζ	Asp_{P7}:$O^{\delta1}$	0.410	44
Arg_{L94}:$N^{\eta1,2}$	Asp_{P7}:$O^{\tau1}$	0.307, 0.412	93
Tyr_{H33}:O^η	Asp_{P7}:$O^{\delta2}$	0.238	2
Lys_{H56}:N^ζ	Asp_{P7}:$O^{\delta2}$	0.295	55

Percentage of frames (out of total number of trajectory frames) is given.
[a] Donor–acceptor distance found in the crystal structure of antibody with the linear peptide.

3.2 RMSF and RMSD analysis

Figure 1 shows the RMSF of C^α atoms as calculated from the free and bound trajectories for the $T_1SSPSAD_7$ peptide. The peptide showed only limited mobility in the bound

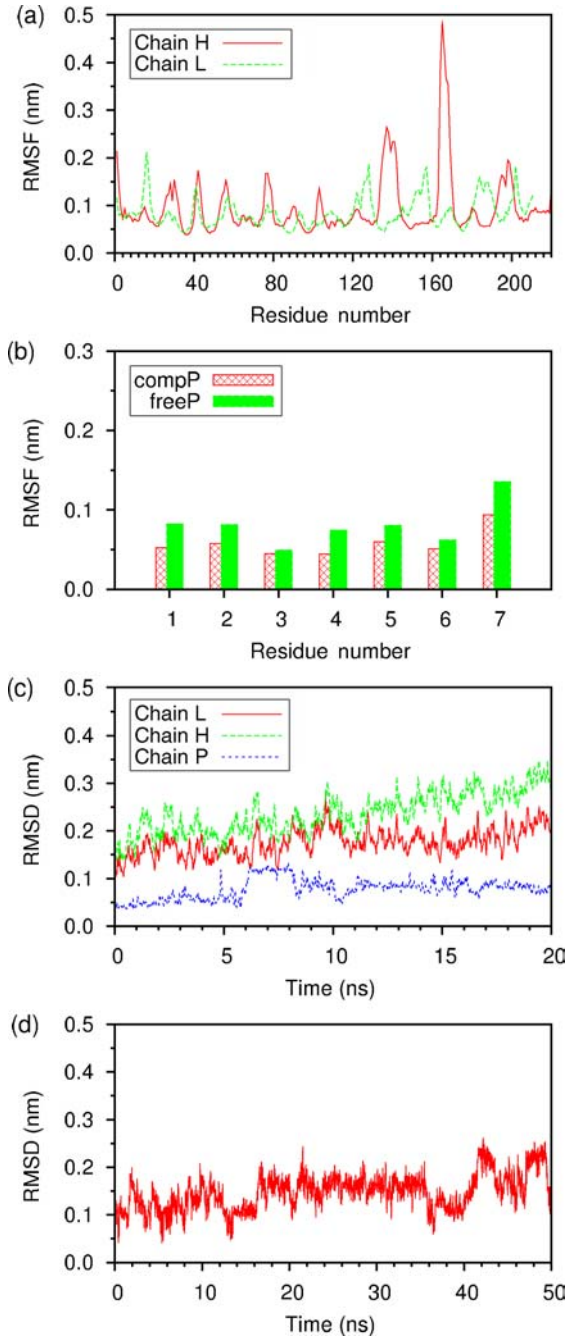

Figure 1. (a) RMSF of C^α atoms of antibody's heavy (H) and light (L) chains, (b) RMSF of peptide's C^α atoms in bound (compP) and free (freeP) states, (c) time evolution of backbone atoms (N, C^α, C′, O) RMSD during the compP trajectory of antibody's heavy (H) and light (L) chains and peptide (P) and (d) time evolution of backbone atoms (N, C^α, C′, O) RMSD during the freeP trajectory of the peptide. For interpretation of colours in this figure the reader is referred to the online version of this article.

state and remarkably small RMSF values (<0.1 nm) confirmed this fact. This also illustrates the goodness of fit of the peptide into the antibody's binding site.

Most of the 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 0.5 nm. For example, the RMSF peak at 0.4 nm corresponds to residue 165 of the heavy chain. A similar situation has been observed with the simulation of the linear peptide complexed with the CAMPATH-1H antibody [12]. Residues of the CDRs that were found to have contacts with the peptide antigen showed minimal RMS fluctuations between 0.05 and 0.1 nm (Figure 1). This fact indicates that the conformation of the CDRs was not influenced by the cyclisation of the peptide and that interactions between antibody and peptide remained almost intact. Small RMSF fluctuations are indicative of minimal conformational mobility and the minimal effect that the cyclisation of the peptide had on antibody's conformation is corroborated by the small RMSF values of the CDR regions, as well as by the similarity of the RMSF profiles during the current and previous (with the linear peptide [12]) simulations.

RMSF values of peptide's C^{α} atoms of the MD trajectory of peptide in bound state (compP) were found between 0.06 and 0.1 nm (Figure 1). These low RMSF values underline the conformational rigidity of the peptide in the bound state. In the free state (freeP trajectory), RMSF values of peptide's C^{α} atoms were found between 0.05 and 0.15 nm. Although a raise in RMSF values is expected when the peptide is simulated in the unbound state (freeP trajectory), the difference in RMSF values is relatively small. For example, the corresponding RMSF values ranged between 0.15 and 0.28 nm during the simulation of the linear peptide [12]. The decreased RMSF values illustrate the effect of cyclisation on peptide's structure. The Asp_7 residue, located at the C-terminal part of the peptide, showed the highest RMSF values in both compP and freeP trajectories (0.1 and 0.15 nm, respectively). On the other hand, the Ser_3 residue showed the smaller RMSF value in both compP and freeP trajectories with values close to 0.05 nm in both cases. In general, RMSF values of peptide's C^{α} atoms were found with remarkable covariance in comp and freeP trajectories (Figure 1).

The antibody's heavy and light chains showed moderate mobility during the MD trajectory, as revealed from backbone RMSD time series plot (Figure 1). RMSD (values in parentheses indicate standard deviation) of the heavy chain exhibited values between 0.13 and 0.34 nm which averaged at 0.23 nm (0.04 nm). The 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 relatively stable through the simulation time, which indicates the stability of the trajectory. However, during the last 10 ns of the compP trajectory there is a small gradual increase in the RMSD values of the antibody's heavy chain. The observed values are within an expected range in a MD simulation, but there is some evidence of conformational change in the heavy chain.

The peptide's backbone atoms RMSD time evolution (in the bound state, Figure 1(c)) showed limited fluctuation and recorded values were found to be between 0.1 and 0.26 nm. The time series average value was found to be 0.21 nm (0.016 nm). These relatively low values, along with the previously analysed RMS fluctuations, indicate the very low flexibility of the peptide when bound to the antibody's binding site. A trajectory movie (Supplementary material) also provides visual evidence about this fact.

Much like 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 (Figure 1(d)). Backbone RMSD values of the peptide ranged between approximately 0.05 and 0.25 nm during the 50 ns trajectory. These values averaged at 0.16 nm (0.05 nm). RMSD fluctuation remained relatively stable during MD trajectory. In line with the RMSF analysis, RMSD analysis indicates that the peptide remained closed in its initial conformation, which was actually that found in the crystal structure. If the results of MD of the linear peptide in the free state are taken into consideration, where RMSD values reached 0.4 nm, it is assumed here that disulphide cyclisation not only reduced the conformational space available to the peptide but also resulted in 'fixing' the conformation close to those found in the bound state.

From this point of view, analysis of the freeP trajectory corroborates the initial design hypothesis of this work that substitution of Thr_1 and Ala_6 by Cys residues and disulphide cyclisation will 'freeze' the conformation of the peptide. This can be seen in Figure 2, where representative

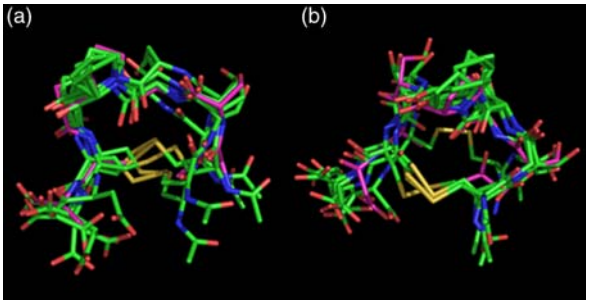

Figure 2. (a) Five snapshots of peptide's structure from the compP trajectory (every 4 ns) and (b) five snapshots of peptide's structure from the freeP trajectory (every 10 ns). Peptide frames are superimposed on the backbone atoms of the corresponding atoms from the crystal structure (in magenta). Hydrogen atoms are omitted for the clarity of representation. For interpretation of colours in this figure the reader is referred to the online version of this article.

structures of the cyclic peptide from compP and freeP trajectories are depicted.

3.3 Backbone conformation and secondary structure of the peptide antigen

The initial (crystal structure) conformational state of the $T_1SSPSAD_7$ peptide was found as CCTTTTC according to STRIDE assignment. A type I β-turn around the Pro_4Ser_5 existed in the central part of the peptide. Previous MD studies showed that this β-turn remains stable when the peptide was simulated in the complex state, but disappeared when simulated in the free (unbound) state. Disulphide cyclisation, after substitution of Thr_1 and Ala_6 with cysteine residues, is expected to stabilise this β-turn. Trajectory analysis of the peptide's secondary structure revealed that this conformation was retained for 82% of the frames, during the compP trajectory. Analysis of the freeP trajectory revealed two main conformational clusters (Table 2) with secondary structure assignments CCT-TTTC and TTTTTTC (percentage of occurrence was 45 and 43%, respectively). Although the turn conformation of the central part of the peptide was retained in both conformational clusters, a second turn appeared at the N-terminal part of the peptide. Overall, both trajectories showed remarkable similarity in secondary structure assignments, which corroborates the idea that disulphide cyclisation can stabilise the solution conformation of the peptide close to the bound structure of the linear precursor peptide. Of course, even after cyclisation the peptide can access bigger conformational space in free state than in complex state. But the fact that both trajectories showed similar secondary structures illustrates the possible success of this molecular design.

The β-turn structure observed at the Ser_3-Pro_4-Ser_5-Ala_6 peptide's fragment was very well conserved during both trajectories. However, the initial type I particular conformation was only retained during the compP trajectory for 57%. Another 25% of the trajectory frames were found with type IV β-turns. Thus, type I was the dominant conformation, but a significant part of the trajectory escaped to slightly different backbone conformation. During the freeP trajectory, the type I β-turn of the Ser_3-Pro_4-Ser_5-Ala_6 peptide's fragment was purely conserved; it appeared for only 20% of the frames. Ramachandran plots of backbone dihedrals (Figure 3) demonstrate that it is basically due to Ser_5's ψ dihedral angle. However, the fluctuation of ψ_5 did not result in total disappearance of the β-turn. The Ser_3-Pro_4-Ser_5-Ala_6 fragment retained in β-turn conformation (for total 91% of the simulation time) but type IV (42%) and type VIII (29%) also appeared. The freeP trajectory revealed another β-turn formation at the Cys_1-Ser_2-Ser_3-Pro_4 fragment, that did not exist at the initial conformation. For 47% of the simulation time (Table 3), this fragment was found in either type IV of type VIII β-turn conformation. As shown in Table 3, both turns co-existed

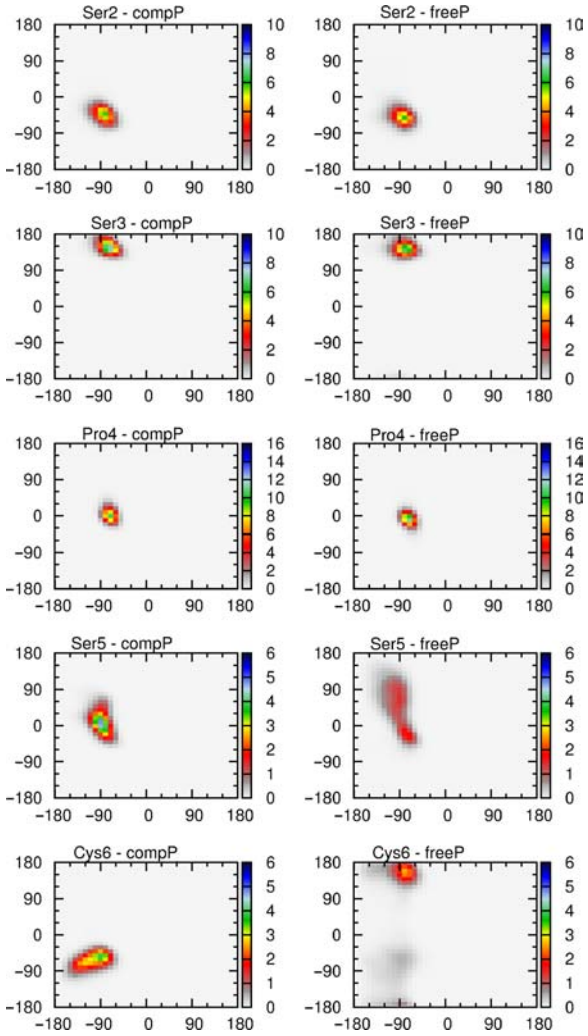

Figure 3. Ramachandran map (backbone ϕ/ψ dihedrals) for peptide's residues in the bound (compP) and free (freeP) trajectories. Horizontal axis is for ϕ angle and vertical axis is for ψ angle. Two dimension probability density plots obtained are shown; z-axis is the percentage of frames found within 10° dihedral angle bin. The adjacent colour bar is used to identify regions of low (grey) versus high (blue) populations. For interpretation of colours in this figure the reader is referred to the online version of this article.

Table 2. Main conformational clusters after secondary structure assignment of the compP and freeP MD trajectories.

	Trajectory	
Secondary structure	compP	freeP
CCTTTTC	82	45
TTTTTTC	2	43
CCCCCCC	14	7

Percentage of frames is given.

Table 3. β-turn occurrence during the two MD trajectories.

		Trajectory	
Fragment	Type	compP	freeP
Cys1-Pro4	VIII		21
	IV		28
Ser3-Cys6	I	51	31
	VIII		25
	IV	18	30
Pro4-Asp7	I	4	
	IV	78	

Percentage of frames is given.

for approximately 43% of the time, nearly half of the trajectory.

Analysis of intrapeptide hydrogen bonds during the compP trajectory showed that the Ser_3:O-Ala_6:N distance and the Ser_3:O-Ala_6:H^N-Ala_6:N angle satisfied the hydrogen bond criteria for only 23% of the trajectory time. Thus, the hydrogen bond that stabilised the β-turn of the central part of the peptide was only poorly conserved. The corresponding percentage of frames was 45% when the linear peptide simulated in the bound state [12]. Although, the existence of a hydrogen bond is not an absolute requirement for the acceptance of a β-turn structure, it is clear that the stabilisation of the β-turn due to the existence of the intramolecular hydrogen bond was scaled down after the imposed disulphide bond. On the other hand, a new hydrogen bond appeared between Ser_3:O and Ser_5:N atoms, which corresponds to a γ inverse turn. This interaction appeared for approximately 65% of the time. Thus, the bulge in the central part of the peptide fragment was not completely lost, but it was somewhat altered in comparison with the initial crystal structure. Despite the disruption of the canonical hydrogen bond interaction, the fragment Ser_3-Pro-Ser-Cys_6 remained in β-turn structure, as measured by Ser_3:C^α-Cys_6:C^α distance the Ser_3:C^α-Pro_4:C^α-Ser_5:C^α-Cys_6:C^α dihedral angle, for approximately 80% of the time.

Simulation of the peptide in the free state (freeP trajectory) showed that the initial turn conformation was retained for 85% of the time (Table 3). The initial CCTTTTC conformational state was found in 82% of the frames in the compP trajectory and 45% in the freeP trajectory. Compared with 5% of the CCTTTTC conformation of the linear peptide, found in a previous research, 45% of the frames demonstrates the effect of the cyclisation in the conservation of the turn structure. From this point of view, disulphide cyclisation succeeded in its purpose: it offered a structural framework of β-turn stabilisation.

3.4 Antibody–antigen interactions

The binding site of the CAMPATH-1H antibody is highly basic. A number of polar hydrogen bonds between the peptide and the antibody have been reported to contribute to the stability of the complex. However, several of these hydrogen bonds were poorly conserved in the compP trajectory (Table 1).

One of the consequences of the disulphide cyclisation of the peptide antigen was the loss of a hydrogen bond interaction between Glu_{101H}:$O^{\varepsilon 2}$ and Cys_{1P}:S^γ side chain atoms. The original distance of 0.24 nm between these two atoms fluctuated between 0.29 and 0.86 nm during compP trajectory. The substitution had also some effect on the nearby residue. Hydrogen bond between Ser_{2P}:N and Ser_{2P}:O^γ was conserved for only 30% of the compP trajectory.

Ser_{3P} and His_{81L} were found in hydrogen bond state via their side chains. The Ser_{3P}:O^γ-His_{91L}:$N^{\delta 1}$ distance ranged between 0.252 and 0.60 nm during com-MD trajectory. The corresponding distance in the X-ray structure was 0.264 nm. Trajectory analysis showed that these two side chains remained in hydrogen bond state for 56% of the simulation time. It should be noted here that the hydroxyl group of Ser_{3P} acted as an acceptor and imidazolium group of His_{91L} acted as a donor of this hydrogen bond. Arg_{96L}'s side chain also contributed a hydrogen bond to Ser_{3P}'s side chain for 17% of the simulation time. The Arg_{96L}:$N^{\eta 2}$-Ser_{3P}:O^γ distance ranged between 0.287 and 0.886 nm during com-MD trajectory, while the corresponding distance in the X-ray structure was 0.30 nm. Thus, the side chain of Ser_{3P} remained in hydrogen bond state with CDR-L3 residues for approximately 73% of the simulation time.

Pro_{4P}, lying at the centre of the peptide's sequence, dominated the hydrophobic interactions with the antibody. Pro_{4P}'s side chain aliphatic atoms were found to make several contacts (distance less than 0.4 nm) with Tyr_{32L}'s (CDR-L1) side chain. Considering the X-ray structure of the complex, nine pairs of interatomic distances of less than 0.4 nm between Pro_{4P}'s and Tyr_{32L}'s side chain carbon atoms were found. Of course, not all of them were conserved during com-MD trajectory. However, seven of them (not at the same necessarily) were found to exist (for at least 20% of the trajectory time) in a distance less than 0.4 nm. The escape of the 0.4 nm cut-off distance can be attributed to minor fluctuations of the χ^1 angle of Tyr_{32L}, which was averaged at $-59°$ (7.7°) during com-MD trajectory. While this side chain dihedral did not change substantially during the simulation time, it must be noted that a small fluctuation of 15°, for example, can result in more than 0.05 nm increase in distance between side chain atoms of Pro_{4P} and Tyr_{32L}.

During the second half of the compP trajectory, Pro_{4P}:O and Arg_{L94}:$N^{\eta 2}$ atoms were found in hydrogen bond state (for 47% of the total simulation time). The original distance (X-ray) of these two atoms was 0.68 nm. This fact indicates that a medium sized conformational change occurred at the CDR-L3 region that probably stabilised the complex. Thus, some new bonds can be formed in place of the broken ones.

Ser_{5P} dominated hydrogen bond interactions between peptide and antibody. In the original, X-ray structure, three hydrogen bonds were found between peptide and antibody. Namely, Ser_{5P}:O-Arg_{94L}:$N^{\eta 1}$ with distance 0.3 nm, Ser_{5P}:O^{γ}-His_{91L}:O with distance 0.263 nm and Ser_{5P}:O^{γ}-Arg_{96L}:N^{ε} with distance 0.284 nm. This means that Ser_{5P}:O^{γ} atom acted both as hydrogen bond donor (with His_{91L}:O as acceptor) and acceptor (with Arg_{96L}:N^{ε} as donor). The initial Ser_{5P}:O-Arg_{94L}:$N^{\eta 1}$ hydrogen bond was retained for only 29% of the compP MD trajectory. However, another hydrogen bond between Ser_{5P}:O and Arg_{96L}:$N^{\eta 2}$ atoms, not present in the initial structure, acted in a complementary mode and existed for 41% of the simulation time. The hydrogen bond between Ser_{5P}:O^{γ} and Arg_{96L}:N^{ε} atoms was retained for 87% of the simulation time during com-MD trajectory, while hydrogen bond between Ser_{5P}:O^{γ} and His_{91L}:O was retained for 61% of the simulation time.

Finally, a severe loss of the hydrogen bonding occurred at the C-terminal of the peptide. Due to the basic nature of antibodys' binding sites, numerous hydrogen bonds and salt bridges existed between the Asp_{7P} residue and antibodys' basic residues. With the exception of Asp7P-Arg94L, all other interactions were significantly suppressed or extinguished. For example, a very strong hydrogen bond between Arg_{H52}:N^{ε} and Asp_{P7}:$O^{\delta 1}$ atoms was completely lost during compP trajectory.

3.5 *Configurational entropy*

One of the main objectives of the current work was to examine to what extent the cyclic peptide accesses the same conformational space in complex and free state. As it is expected, conformational fluctuation of peptide in the bound state can be rather prohibited. On the other hand, the imposed cyclisation through disulphide bond formation prohibits the opening of the turn conformation to an extended one. Thus, it is assumed that the cyclic peptide should share (at least partly) the same conformational space in both free and bound states facilitating the antibody binding of the peptide due to reduction of the entropic cost. The similarity of the peptide's structure in the bound state with the crystal structure of its linear precursor has been already discussed.

Similarity of the peptide structures obtained from freeP and compP MD trajectories can be seen in Figure 2. However, configurational entropy offers a more quantitative way to estimate the difference or similarity or the overlap of the trajectories. As it can be seen from Figure 4, the peptide sampled similar conformational spaces in both the compP and freeP trajectories. However, sampling during compP was only a subset of the conformational space sampled during freeP trajectory. This is something to be expected because of the restriction to flexibility that the antibody imposes to the peptide. On the other hand,

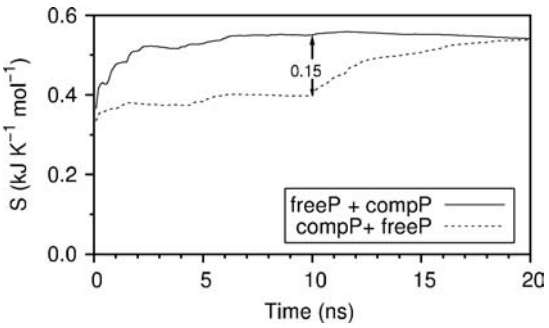

Figure 4. Plot of estimated entropies of backbone atoms of the $(S,S)C_1SSPSCD_7$ peptide. Frames from the freeP were appended to the compP trajectory and vice versa. Last 10 ns from the two trajectories were used, assuming equilibrium was reach. See Section 2 for details of calculation.

the difference in configurational entropy was estimated to be 0.15 kJ/K/mol, a relatively moderate value, indicating that the peptide's conformation in free and bound states do not differ dramatically. From this point of view, the design of the cyclic peptide was successful: 'active' conformation was retained in the solution for 50 ns, which is not a negligible time. Thus it can be expected that the antibody could bind the peptide from its solution conformation with less entropic cost. But despite the structural similarity, is this really 'active' conformation?

3.6 *BSA calculations*

The connection between thermodynamics of protein/ligand association and antibody/antigen complex formation and BSA is well described in the literature [47–49]. Figure 5 shows the time evolution of BSA at the interface between antibody and peptide over simulation time as calculated according to Equation (3) from the compP trajectory. Time series of BSA ranged between

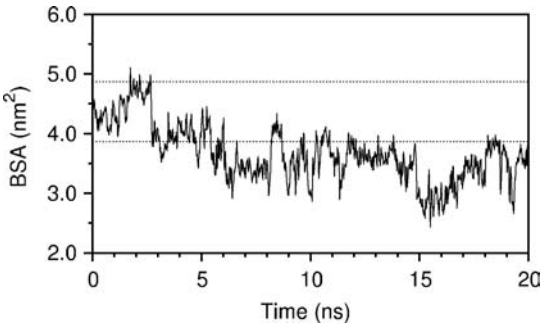

Figure 5. Time series of non-polar buried surface area (BSA) between peptide and antibody from the compP trajectory. See Section 2 for details of calculation. The dashed horizontal line at 4.87 nm^2 indicates the experimental (X-ray) value of BSA of the crystallised linear analogue [11] while the dashed line at 3.87 nm^2 indicates the BSA value reduced by 1 nm^2. This is to emphasise the fact that the great majority of the frames were found with significantly reduced BSA values.

2.13 and 5.50 nm^2 and averaged at 3.67 nm^2 (std 0.50 nm^2). The BSA of the starting conformation was estimated to be 4.87 nm^2. From these values, it can be roughly estimated that the BSA between peptide and antibody significantly decreased through the MD trajectory. The majority of the trajectory frames (69%) had a BSA less than 3.87 nm^2, a value that is 1 nm^2 below those found in the crystal structure. It must be noted that variations of the BSA during MD trajectories are to be expected because of the dynamic nature of the protein structure. Moreover, a recent study [50] questioned the precision of the calculated solvent-accessible surface area of proteins, and an error of 0.5–1 nm^2 in the calculation of BSA of protein/protein complexes has been suggested. But even with findings taken into consideration, the difference in experimental values (X-ray) and the average value of the MD trajectory ($4.87 - 3.67 = 1.2\,nm^2$) is far from what is considered a measurement error. The loss of the ~25% (1.2/4.87) of the BSA between peptide and antibody cannot be neglected, and it is concluded that the affinity of the antibody for the peptide has been significantly reduced after the disulphide cyclisation.

As it can be seen from Figure 5, a sharp fall in the value of BSA can be observed at approximately 2.5 ns of the simulation time. Mean value of BSA calculated during the last 17 ns (omitting the first 3 ns) was found as only 3.53 nm^2. These observations underline the importance of sufficient sampling during MD simulations. Short runs (1–2 ns) can produce misleading results and should be avoided.

It has been proposed recently [47] that the free energy of protein/ligand binding can be estimated by the empirical formula:

$$\Delta G^0_{\max} \approx -30 - \frac{\mathrm{CSA}_{\mathrm{apolar,bindingsite}}}{10}\,\mathrm{kJ/mol}, \qquad (8)$$

where CSA (Connolly surface area) is the non-polar accessible surface area. The parametrisation of the Equation (8) has been performed with Connolly method of surface area calculation, but if we take into consideration that 1 kJ/mol equivalents with 20 $Å^{-2}$ in Connolly's methods and $37 \pm 7\,Å^{-2}$ in Lee–Richards method (NACCESS) then we can use the above formula with appropriate scaling. For the initial complex (X-ray), $\Delta G^0_{\max}$ was calculated −56.3 kJ/mol. Using the average trajectory value of 367 $Å^{-2}$ of the ASA of antibody/peptide complex, which scales to 198.4 $Å^{-2}$ of CSA, the value of −49.8 was estimated for the $\Delta G^0_{\max}$ of the antibody/cyclic peptide. Thus, it is estimated that the difference in the Gibbs free energy of binding between the linear and cyclic peptides is 6.5 kJ/mol. Although the results should be interpreted with care due to intrinsic limitations of such calculations, it is clear that reduction in BSA between antibody and peptide resulted in lower binding affinity.

4. Concluding remarks

Molecular modelling techniques coupled with extensive MD simulations in explicit solvent have been utilised in order to design and computationally validate the effect of disulphide bond formation on a peptide that binds to the CAMPATH-1H antibody. As cyclisation is routinely used in order to enhance binding properties and/or biological activity of bioactive peptides, the initially proposed molecular model seemed quite a reasonable guess: the T_1SSPAP_7 peptide could be replaced by the $(S,S)C_1SSPCP_7$ one, given the proximity of Thr_1 and Ala_6 side chains. After the initial building and molecular mechanics calculations, the stability of the antibody/peptide complex as well as conformation of the peptide in both bound and free states was explored with extensive MD simulations in explicit water.

Analysis of the results showed that the proposed model is quite promising in order to replace the linear one as a peptide mimotope. The peptide remained stable into the binding groove of the CAMPATH-1H antibody and experienced only moderate fluctuation around the initial structure. Moreover, the initial conformation of β-turn around the Pro_4Ser_5 fragment was perfectly conserved during the 20 ns MD study in complexed state and 50 ns in free state.

The study of the cyclic peptide in the free state revealed also interesting characteristics of its conformation. The results corroborated the hypothesis that the engineering of the peptide and disulphide bond formation can substantially enhance peptide's conformational stability in water. The β-turn around the Pro_4Ser_5 fragment was retained for 91% of the time (50 ns). On the contrary, a previous study showed the complete abolishment of turn conformation of the linear peptide in solution [12]. The adoption of a well-structured conformation of the peptide in solution is thus attributed to the cyclisation.

Estimation of configurational entropy profiles of the peptide in free and bound states was used in order to extract information about the similarity of the conformational space accessed. A heuristic formula that uses combined trajectories of the two separate simulations was used. Results indicated that the two MD trajectories overlap and they sampled common areas of the conformational space. More specifically, the compP trajectory sampled a subspace of the conformational space of sampled by the freeP trajectory.

The peptide showed similar conformational properties in both free and bound states. But a significant reduced tendency for hydrogen bonding interactions between the peptide and antibody was observed. It seemed that the antibody lost (at least partly) its affinity to bind the peptide. Analysis of the BSA between the peptide and

antibody showed that 25% of the contact surface was lost. This probably has severe effects in the antibody's affinity for the peptide. So, while the cyclisation of peptide results in solution conformation that retains most of peptide's conformational characteristics, it did not help the antibody bonding.

While cyclic peptides are still a very promising replacement of their linear analogues, their usage in computer-aided design should be carefully implemented and their conformational rigidity should not be over-emphasised. The current study presents results that has two sides: (1) disulphide cyclisation effectively reduced the flexibility of the linear peptide and transformed a complete unordered structure to a quite stable β-turn conformation and (2) minor changes in antigen/antibody contacts resulted in gradual loss of several interactions that eliminated the BSA by approximately 25% and thus reducing the binding affinity.

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