Conformational Studies of the 313-320 and 313-332 Peptide Fragments Derived from the α_{IIb} Subunit of Integrin Receptor with Molecular Dynamics Simulations

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Abstract The peptide sequence YMESRADRKLAEVG-RVYLFL, derived from 313-332 region of the α_{IIb}, has been identified as a potent inhibitor of platelet aggregation and fibrinogen binding to $\alpha_{IIb}\beta_3$. More detailed studies have revealed that the $Y_{313}MESRADR_{320}$ sequence is the shortest octapeptide with strong inhibitory activity. This work provides insight of the solution conformation of these peptides, by performing extensive molecular dynamics simulations of 100 ns. The 8mer peptide has no stable conformation in water while the 20mer peptide retains a relative conformational stability. Analysis of side chain orientation of the RAD fragment revealed the synplanar arrangement of guanidinium and β-carboxylic groups providing a framework for explaining the similar biological activity of the two peptides, despite their differences in sequence and conformation.

Keywords Computer simulation · Integrin · Molecular dynamics · Peptide conformation · Platelet aggregation inhibition · SAR · α_{IIb}

Introduction

Integrins are receptors that mediate many biological important processes (Humphries 2000). They are heterodimers which come into non-covalent association and they are composed by two membrane proteins. The integrin $\alpha_{IIb}\beta_3$ (glycoprotein IIb/IIIa) has a very significant role in thrombosis and haemostasis. It mediates interactions between

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platelets and several ligands, primarily fibrinogen (Phillips et al. 1988). Integrins are well known for their recognition of protein ligands through the RGD sequence (Salsmann et al. 2006; Takada et al. 2007). Crystallization of integrin segments with RGD ligands has revealed important futures of the binding mode (Xiao et al. 2004; Xiong et al. 2002), such as binding to the α subunit β propeller exposed interface. One Mg^{2+} ion participates in integrin binding of RGD peptides (Craig et al. 2004; Xiao et al. 2004) by coordinating to the Asp side chain, while guanidium group of Arg side chain binds to carboxylic side chains of Asp residues located at the α subunit β propeller domain (Xiong et al. 2002).

Recently, the sequence of the α_{IIb} subunit has been mapped with 20mer peptides for identifying fibrinogen binding domains (Biris et al. 2003a). The most promising region, thus the 313-332 fragment, was further investigated (Mitsios et al. 2004, 2006), in order to define important residues for its activity and minimum chain length that can retain the inhibitory and aggregatory capability. These investigations revealed that the peptide sequence $Y_{313}MESRADR_{320}$, located at amino terminus of the initial 20mer, is a putative fibrinogen-binding region by inhibiting platelet aggregation and by antagonizing fibrinogen association (Mitsios et al. 2004). Biological activity of the peptide was attributed primarily to the (highly conserved in the integrins) RADR motif (Mitsios et al. 2006; Stanica et al. 2008). Interestingly it was found that the peptide lost its inhibitory activity after deletion of the amino terminus YMES sequence, indicating that the carboxyl terminus RADR motif is not capable to act independently from the rest of sequence, despite its similarity with the well known RGD motif. On the other hand, alanine scanning of the $Y_{313}MESRADR_{320}$ sequence revealed that substitution of each of the Y_{313}, M_{314}, E_{315} or S_{316} residues does not affect biological activity (Stanica et al. 2008). Inhibitory activity

has also diminished after elongation of the Y_{313}MES-RADR$_{320}$ sequence from both amino and carboxyl terminus. Since there is no structural information for the peptide, these observations where ascribed to the strand conformation of the residues surrounding the RADR fragment, as found in the crystal structure integrin/fibrinogen complex (Xiao et al. 2004).

This work presents a computer simulation study of the aqueous conformation of the Y_{313}MESRADRKLAEVG-RVYLFL$_{332}$ and Y_{313}MESRADR$_{320}$ peptide sequences derived from the α_{IIb} integrin subunit. State of art molecular dynamics protocols have been applied in order to elucidate the most important characteristics of their conformational and dynamical properties. Molecular dynamics 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 (Hansson et al. 2002; Karplus and McCammon 2002; Morikis and Lambris 2004; van Gunsteren and Berendsen 1990; van Gunsteren et al. 2008). Similar computational approaches have been showed to add valuable information to existed experimental data of antibody/antigen complexes (Lorenzo et al. 2007; Tatsis et al. 2009b; Voordijk et al. 2000), to explore the dynamics of peptide/MHC interactions (Painter et al. 2008; Wan et al. 2004; Yaneva et al. 2009; Zacharias and Springer 2004), in searching for active conformational templates (Stavrakoudis et al. 1997, 2003; Tatsis et al. 2006, 2008), to establish a model of protein/peptide dimerization based on a disulfide bridge (Stavrakoudis 2009b), to analyze hydrophobic clusters of protein structures (Espinoza-Fonseca 2009; Tatsis et al. 2009), to analyze the non-bonded interactions that stabilize secondary structures in peptides (Santiveri et al. 2004; Stavrakoudis et al. 2009; Wang and Sung 1999; Yoda et al. 2007), or to explore pathways of protein folding (Duan and Kollman 1998; Karplus and Sali 1995; Morra et al. 2008). Atomistic simulations of peptides provide an excellent framework to study the intrinsic flexibility of peptides and to describe the non-bonded interactions that contribute to peptide's structure and/or function, something that is not always accessible with other experimental methods (Hansson et al. 2002; van Gunsteren and Berendsen 1990; Voordijk et al. 2000).

Methods

Molecular mechanics and/or dynamics simulations were performed with NAMD (Phillips et al. 2005). The coordinates of the initial structure of Y_{313}MESRADR$_{320}$ (from this point on P1 or 8p) and Y_{313}MESRADRKLAEVG-RVYLFL$_{332}$ (from this point on P2 or 20p) peptides were extracted from PDB's (Berman et al. 2000) entry 1txv (Xiao

et al. 2004). Hydrogen atoms were added with the autopsf utility of the VMD program (Humphrey et al. 1996). The peptides were solvated with TIP3P (Jorgensen et al. 1983) water molecules in an orthogonal parallelipipedon box, leaving a distance of at least 1.8 nm of any peptide atom to the edge of the solvent box. The final dimensions of the boxes were $4.98 \times 5.94 \times 4.65$ nm^3 in the P1 case and $5.68 \times 7.46 \times 5.02$ nm^3 in P2 case. Ten pairs of Na$^+$ and Cl$^-$ counter ions where added in the box containing the P1 peptide. Fourteen Na$^+$ and fifteen Cl$^-$ ions where added in the P2 box. Both P1 and P2 systems where treated similarly from this point on. In the first step, energy minimization was performed for 2,500 steps under PBC conditions. The non-bonded pair list was updated every 10 steps, while non-bonded interactions were computed every second step. Long range electrostatics were computed with the PME method (Darden et al. 1993; Toukmaji and Board 1996) every second step, with a grid spacing of less than 0.1 nm. Bonds to hydrogen atoms were constrained with the SHAKE (Barth et al. 1995; van Gunsteren and Berendsen 1990) algorithm, with a relative tolerance of 10^{-8}, allowing a 2 fs step during subsequent MD runs. Heavy atoms of the peptide were restrained to initial coordinates with a force constant of 50 kcal Å^{-1} mol^{-2}. After minimization, the temperature of the system was gradually increased with Langevin dynamics using the NVT ensemble to 310 K during a period 3,000 steps, by stepwise reassignment of velocities every 500 steps. Simulation was continued until 10^5 steps. After this point, the force constant of heavy atoms restraint were reduced to 5 kcal Å^{-1} mol^{-2}, and the simulation was continued for another 10^5 steps. Equilibration was finalized with 2×10^5 steps of Langevin dynamics under NVT conditions by totally eliminating the positional restraints. The simulation was continued under constant pressure, with Langevin piston method (Feller et al. 1995), thus NPT ensemble, for 100 ns (5×10^7 steps). Pressure was maintained at 1 atm and temperature was kept at 310 K. Structures were saved to disk every 500 steps (1 ps) for further analysis.

Conformational Analysis

Conformational analysis and visual inspection of structures were performed with VMD (Humphrey et al. 1996), Carma (Glykos 2006) and Eucb (Tsoulos and Stavrakoudis 2009) software packages. Appropriate circular statistics were taken into consideration for calculation of descriptive values of dihedral angles (Döker et al. 1999). Trajectory analysis of hydrogen bonds were 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 (Frishman and Argos 1995). Structural figures were prepared with PYMOL (Delano 2002).

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 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.

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

(1)

Secondary structure assignment of trajectory frames was performed with the STRIDE program (Frishman and Argos 1995). STRIDE assigns one letter code for each residue of a protein sequence, so E stands for strand conformation, T stands for turn, C stands for coil and B stands for bridge. Hydrogen bonds assignment was based on geometrical criteria: Donor–Acceptor distance less than 0.33 nm and Donor-Hydrogen-Acceptor angle greater than 120°. β-turns were defined by the $C^\alpha(i)$–$C^\alpha(i+3)$ distance to be less than 0.7 nm and the $C^\alpha(i)$–$C^\alpha(i+1)$–$C^\alpha(i+3)$–$C^\alpha(i+3)$ dihedral to be less than 90° (absolute value). Root mean square fluctuation deviation (RMSD) measurements were performed after the removal of the global translation/rotation of the trajectory frames. Structural figures were prepared with PYMOL (Delano 2002).

Conformational Clustering

Assignments of secondary structure of the peptide frames with the STRIDE program were stored into a database and conformations of the trajectories were clustered using simple SQL queries.

Entropy Calculations

Schliter's formulation (Schlitter 1993) was used for the calculation of the configurational entropy (S):

$$S_{\text{true}} < S = \frac{1}{2}k_B \ln \det\left[1 + \frac{k_B T e^2}{\hbar^2}\boldsymbol{M}\sigma\right]$$

(2)

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, $\boldsymbol{M}$ 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:

$$\sigma_{ij} = (x_i - \langle x_i\rangle)(y_i - \langle y_i\rangle)$$

(3)

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 A and B) 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 (Hsu et al. 2005). Such trajectories have been derived for the backbone (bb) atoms (N, C^α, C') of the peptide from the last 80 ns of the P1 (8mer) and P2 (20mer) trajectories, taking into consideration the common fragment 314-319. N- and C-terminal residues from the 8mer peptide were excluded from the analysis, due to their increased mobility. Similar analysis has been applied to reveal coverage of conformational space between free and bound trajectories on peptide/antibody binding study (Stavrakoudis 2009a). Both appending sequences were applied resulting in $S_{\text{bb}}^{\text{P1+P2}}$ and $S_{\text{bb}}^{\text{P2+P1}}$ calculations, where the P2 trajectory was appended to the P1 trajectory (P1 + P2) or the P1 trajectory was appended to the P2 one (P2 + P1). 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 (Hsu et al. 2005), briefly described as:

1. S increases after appending one trajectory after to the other, 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 P1 trajectory with respect to the P2 trajectory is:

$$\Delta S_{\text{bb}}^{\text{P2+P1}} \equiv S_{\text{bb}}(\text{P2}) - S_{\text{bb}}(\text{P1})$$

(4)

and the coverage of the P2 trajectory with respect to the P1 trajectory is:

$$\Delta S_{bb}^{P1+P2} \equiv S_{bb}(P1) - S_{bb}(P2) \qquad (5)$$

Results and Discussion

Crystal Structure Analysis

The structure of a_{IIIb} is composed by beta propeller repeats and the 313-332 fragment is found at the surface of the structure (W5 blade of the a_{IIIb} subunit) in an exposed region, connecting the β_2 and β_3 antiparallel β-strands (Xiao et al. 2004). The 313-332 fragment of a_{IIb} (α subunit) has a remarkable position on the crystal structure of integrin (Xiao et al. 2004), Fig. 1. Secondary structure of the 20mer peptide was found in TEEEETTTEEEEECCEEEEEC state, thus mainly in strand conformation. The fragments Met_{314}-Arg_{317} and Lys_{321}-Glu_{324} form two antiparallel β-strands that are connected with two consecutive type IV β-turns consisted by the Ala_{317}-Asp_{320} and Asp_{318}-Arg_{321} fragments. Backbone hydrogen bonds between Val_{325}:N-Tyr_{313}:O, Glu_{315}:N-Ala_{323}:O, Ala_{323}:N-Glu_{315}:O stabilize this β-hairpin conformation.

Convergence of Simulations

The need of good conformational sampling of biomolecular simulations is very well described in the literature (Smith et al. 2002). Here, two long (100 ns) molecular dynamics trajectories have been produced in order to ensure to that the peptides have visited different areas of the conformational space. Moreover, two more checks have been employed in order to estimate the goodness of the conformational sampling during simulation time.

First, cluster analysis (according to secondary structure assignment with STRIDE) revealed that, in the case of P2 (20mer) trajectory that 22 backbone conformations existed with population over 1%, during the whole simulation time of 100 n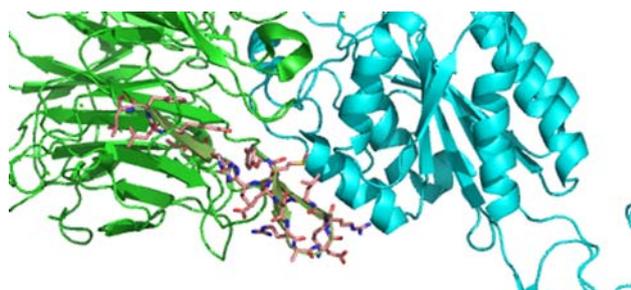s. Splitting the trajectory into two equal parts of 50 ns revealed that there were 23 different conformations in the first part and 21 in the second one (with population over 1%). The results were quite similar, when a 0.5% cutoff was applied: 43 conformations were found in the whole trajectory, while 47 and 45 different conformations were found in the first and second part of the P2 trajectory, respectively. These findings clearly indicate the convergence of the simulation. Similar results were obtained with the P1 (8mer) trajectory, although the second part of the P1 trajectory revealed a somewhat reduced number of conformations in comparison with the first one.

A second check of the simulation convergence was performed with entropic analysis of the backbone conformation of the fragment 314-319 of the P1 (8mer) peptide. N- and C-terminal residues were excluded from analysis due to their increased flexibility. The 100 ns trajectory was split in two parts of 50 ns and the last 30 ns were taken into consideration for entropy calculations, resulting in two (A and B) sub-trajectories. Only backbone atoms were included. Plotting the entropy curves of trajectories $A + B$ (appending B to A) and $B + A$ (appending A to B) is shown in Fig. 2. According to the analysis of Hsu et al. (2005), Figure 2 reveals the overlapping of the two parts of the initial trajectory, as the line of entropy (S) does not show a significant perturbation after the append of trajectory part to the other. This indicates the frame from two distinct parts of the trajectory visited similar conformations, thus the simulation resulted in good sampling of the conformational space. This is something to be expected, as the simulation time was above the usual applied 30–50 ns in peptide simulations (Smith et al. 2002) but this is always a hypothesis until tested with some of the methods proposed be Smith et al. (2002). Evidence provided here corroborate the assumption of conformational convergence and thus provide a solid background for further conformational analysis.
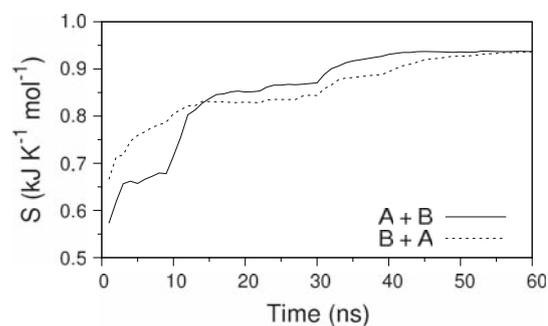

Fig. 1 Location of the 313-332 sequence of the aiib chain of the integrin receptror. PDB's entry 1txv was used. Protein is rendered with *ribbons* and the 313-332 sequence is shown with *sticks*

Fig. 2 Plot of estimated entropy of backbone atoms of the P1 peptide (fragment 314-319). The P1 trajectory was split in two parts (A, B) of 50 ns. The last 30 ns of each trajectory were appended to each other. See Methods for details of calculation

RMSD Analysis

RMSD time evolution of backbone atoms of peptides P1 and P2 is presented at Fig. 3. The initial X-ray structure was used as a reference. Here, it interesting to see the RMSD value of the common fragment (314-319) of the two peptides. As it can be seen, both peptides experienced relatively large deviations from the initial structure with RMSD values in the range of 0.25–4.5 nm. Both peptides escaped from the crystal structure conformation during the equilibration period. Interestingly, the P2 peptide showed relatively lower RMSD values (0.2–0.3 nm) during a period of approximately 60 ns, from the 7th to 67th ns of the simulation time. During the same period the P1 peptide showed increased RMSD values with a peak of 0.45 nm at approximately 24 ns. After the 65th ns of the simulation both peptides showed RMSD fluctuations around 0.2 nm.

Examination of Fig. 3 reveals that the 314-319 fragment of the P2 peptide showed decreased mobility relative to the rest of the molecule and remained closer to the starting conformation of the crystal structure. It is evident also, that the conformation of the 8mer N-terminal part of the P2 peptide is different from the conformation of the P1 peptide which has the same sequence. The loss of the starting conformation of a small 8mer peptide is somewhat expected.
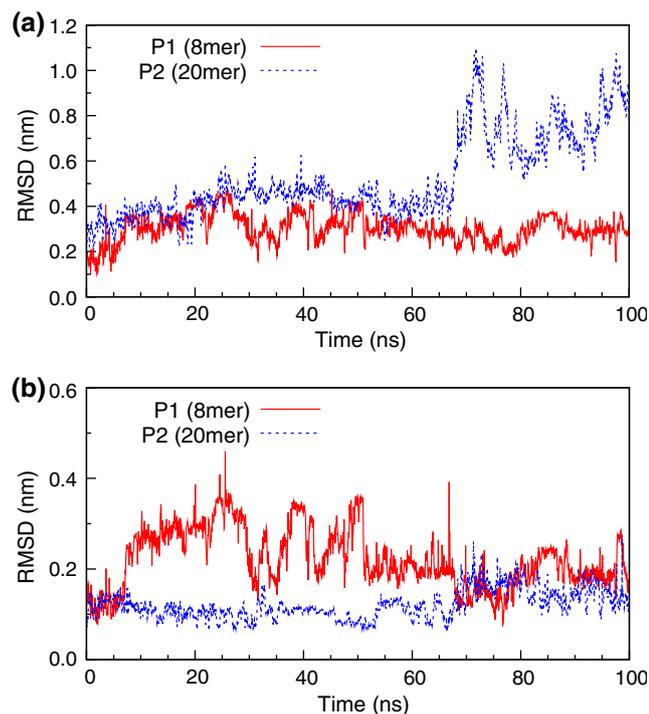

Fig. 3 a Root mean square displacement (RMSD) of the backbone atoms during MD trajectories: P1 for the 8mer peptide, P2 for the 20mer peptide. **b** RMSD time evolution considering only the backbone atoms of the 314-319 fragment of P1 and P2

What is worthy to note is that the same sequence retains considerably its starting conformation when embedded in a bigger peptide sequence. From this point of view, one can say that the conformation of the P2 peptide is closer to the initial structure, regarding the N-terminal sequence, thus this corresponding to the P1 peptide. This implies that shortening a peptide sequence affects drastically the conformation, and the peptide looses even the small stability that might posses. From conformational point of view the two peptides are not equivalent. However, different conformational stability does not necessarily imply loss of activity. It is known for example the P1 (8mer) peptide retain most of the biological activity (Biris et al. 2003a). These data indicate that a receptor can bind a small peptide regardless of its relatively unordered structure, and that that well-defined peptide conformations are not absolute prerequisites for biological activity.

Backbone Conformation and Secondary Structure Analysis

The original strand conformation of 8mer peptide (P1) was lost during equilibration period. Two dominant conformational clusters were observed during P1 trajectory. In a significant part of the trajectory frames (27.9%) all residues were found in coil conformation, without any particular preference for secondary structure stabilization. In the most dominant cluster (39.5% of the trajectory frames, 23.7% of TTTTTT and 15.8% of CTTTTC STRIDE assignment) a β-turn (type IV) was observed in the central part of the peptide. Other small fragments of the P1 peptide showed turn-like conformations for smaller parts of the trajectory. Interestingly, $i \leftarrow i + 3$ main chain hydrogen bonds that could stabilize β-turns were not observed. In all of the cases turn structure that were observed was of type IV.

In the P2 trajectory, as it listed in Table 1, it is evident that the original strand conformation of the 313-320 fragment was much better conserved than in the P1 case. Moreover, the existence of coil conformation in this fragment was found significantly reduced in comparison to the previously analyzed P1 case. On the other hand, 6mer clusters in the P2 case were less populated. The most populated clusters were in CCCCCC and CEETTT states with 16.8 and 16.7% of the trajectory frames, respectively. The fragment $Glu_{315}Ser_{316}$ in particular, was found in strand conformation in many other clusters that were appeared in shorter time during the MD trajectory. From this analysis it is evident the 6mer fragment 314-319 of the P2 peptide remained in a strand conformation, similar to the original conformation for significant amount of the time. The peptide has not showed a compact structure, and its flexibility was also confirmed RMSD analysis. However, it showed

Table 1 Conformational clusters of the 314-319 fragment of the P1 (8mer) and P2 (20mer) trajectories

Conformational states were characterized as Coil (C), strand (E) or Turn (T) with the STRIDE program

#	P1 (8mer) Secondary structure	% Occurrence	#	P2 (20mer) Secondary structure	% Occurrence
1	CCCCCC	27.9	1	CCCCCC	16.8
2	TTTTTT	23.7	2	CEETTT	16.7
3	CTTTTC	15.8	3	TEETTT	9.6
4	CCTTTT	11.0	4	CCTTTT	8.8
5	TTTCCC	7.7	5	CCCTTT	6.4
6	TTTTCC	7.3	6	CCCCCT	4.7
7	TTTTTC	5.1	7	EEETTT	3.4

Table 2 Occurrence of β-turns within P1 (8mer) and P2 (20mer) peptide sequences, as calculated from the corresponding MD trajectories. Percentage of frames that met the geometrical criteria is given

Fragment	P1 (8mer)	P2 (20mer)
$Y_{313}MES_{316}$	33.8	10.7
$M_{314}ESR_{317}$	15.3	4.1
$E_{315}SRA_{318}$	22.4	
$S_{316}RAD_{319}$	37.3	22.9
$R_{317}ADR_{320}$	14.1	37.7
$A_{318}DRK_{321}$		9.6
$D_{319}RKL_{322}$		8.1
$K_{321}LAE_{324}$		6.1
$L_{322}AEV_{325}$		5.5
$A_{323}EVG_{326}$		12.2
$E_{324}VGR_{327}$		10.1
$G_{326}RVY_{329}$		10.3
$V_{328}YLF_{331}$		25.2
$Y_{329}LFL_{332}$		10.4

elements of ordered secondary structure not observed in the P1 case. The diversity and the plethora of the observed conformational transitions allow us to speculate that the time scale of the simulation was big enough to obtain a satisfactory level of sampling different conformations. This is also in agreement with the previously analyzed convergence of the simulations.

Several β-turns were found to exist, although their stability was quite marginal or moderate. Table 2 lists the β-turn assignments in the two trajectories. In the P1 trajectory the main β-turn found was in the $E_{315}SRA_{318}$ fragment (33.8%), while in the P2 trajectory, the main β-turn found was in the $R_{317}ADR_{320}$ fragment (37.7%). The later is in agreement when the β-turn observed in the crystal structure. Thus, the lower backbone RMSD values observed in the P2 trajectory, relatively to P1, considering only the 8mer peptide, can be ascribed to the existence of $R_{317}ADR_{32}$ the β-turn. It is worth to note that the $E_{315}SRA_{318}$ turn was not found during the P2 trajectory. As it also indicated by the Table 1, the N-terminal part of the

P2 peptide (that corresponds to the P1 sequence) was dominated by coil conformation.

Backbone Conformation of the 314-319 Fragment

Conformation of selected frames from the P1 and P2 trajectories are in shown in Fig. 4. It is evident that both peptides visited quite divergent conformational spaces. This is also true if we look at the Ramachandran maps of backbone dihedrals. Figure 5 shows the φ/ψ backbone dihedral angle distribution obtained from the two MD trajectories. As it can be seen, simulation of P1 (8mer) peptide resulted in φ/ψ combinations that scattered in considerable bigger areas than in P2 (20mer) simulation. This fact also corroborates the previous findings about the increased flexibility of the P1 peptide relatively to P2 peptide, and it is in accordance with the secondary structure analysis.

In order to address the relative coverage of the conformational space in P1 and P2 trajectories, the Fig. 6 was prepared. Plotting of entropy S after appending one trajectory to the other clearly indicates that the P2 trajectory covered less conformational space than the P1. Moreover, that conformations sampled during P2 trajectory were within sampled during P1 trajectory, thus the two trajectories overlap but the P1 trajectory contained the backbone conformations of the P2 trajectory. The estimated difference conformational entropy between the backbone atoms of 314-319 fragments was found 87 J^{-1} K^{-1} mol^{-1} (as indicated by the arrow at the middle of the graph).

Salt Bridges and Hydrogen Bonding

Despite the presence of numerous charged groups in the P1 peptide sequence, the conformation of the peptide was not found to be stabilized by salt bridges. The relative stability of the hairpin structure in the P2 case is also evident from hydrogen bond analysis (Table 3). A main chain $Arg_{317} \leftarrow Arg_{320}$ hydrogen bond was observed for 30.1% of the simulation time and provided moderate stabilization
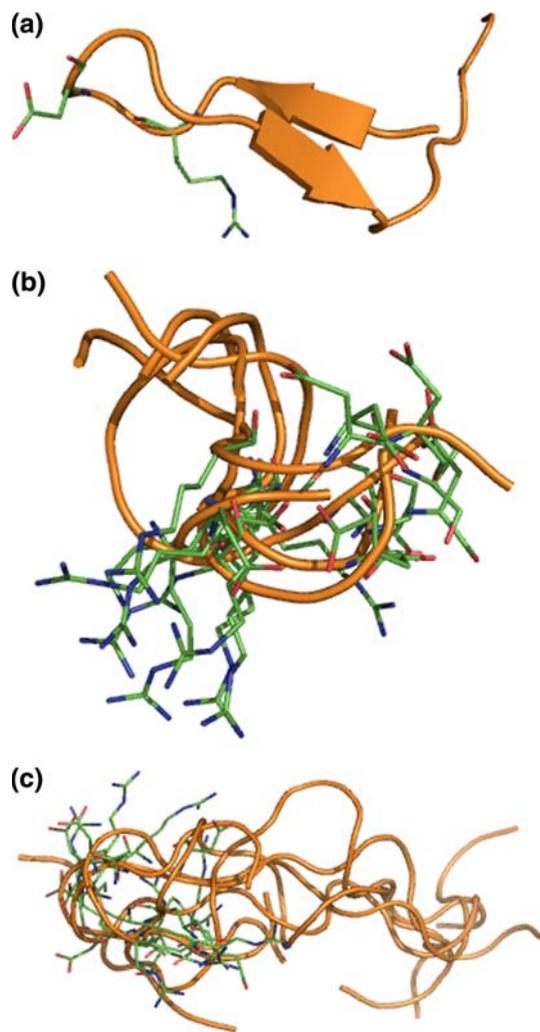
Fig. 4 **a** The P2 (20mer) peptide from the crystal structure, **b** five representative frames (every 20 ns) from the P1 trajectory and **c** five representative frames (every 20 ns) from the P2 trajectory. Backbone conformation is shown with *orange cartoons* while side chains of Arg$_{317}$ and Asp$_{319}$ residues are shown with *sticks*. (Color figure online)

Fig. 5 Distribution of φ/ψ backbone dihedral angles of selected residues (314–319). Two dimension probability density plots were obtained, with φ angle in the horizontal axis and ψ in the vertical one. The adjacent *colour bar* at each graph is used to identify regions of low (*blue*) versus high (*red*) populations (percent of frames in 5° dihedral angle bin search). (Color figure online)

of the β-turn around the Ala$_{318}$Asp$_{319}$ fragment. Tyr$_{313}$ $\leftarrow$ Val$_{325}$, Met$_{314}$ $\leftarrow$ Leu$_{322}$ and Glu$_{315}$ $\leftarrow$ Leu$_{322}$ main chain hydrogen bonds also contributed to the hairpin's stability as they were observed for 15.3, 36.9 and 37.4% of the trajectory frames, respectively.

Overall, the electrostatic interactions did not play critical role in the stability of both peptides. This is somewhat unexpected, considering the abundance of the charged groups in the sequence of the peptides, which they also possessed uncapped N- and C-terminals. The absence of ordered structure in the case of P1 and the limited presence of secondary structural elements of P2 case indicate that electrostatic interactions in aqueous solution cannot dominate peptide's stability, at least in the current case.
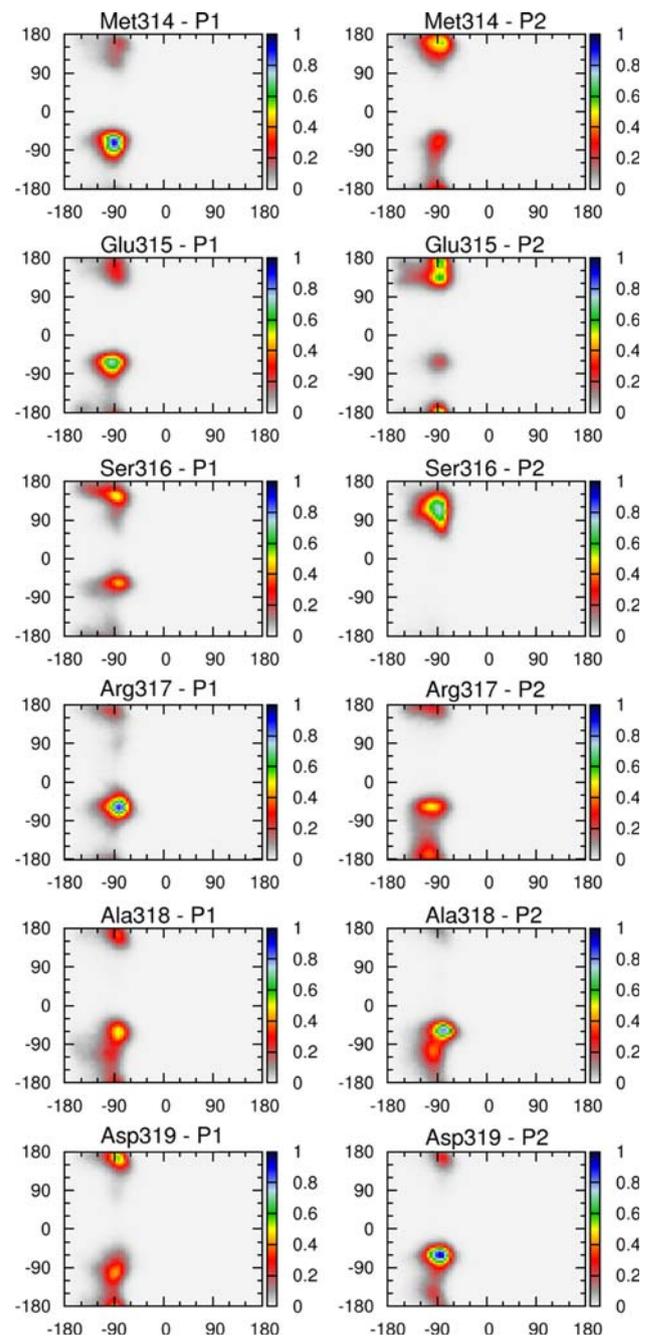
Biological Implications of the RAD Fragment Conformation

It has been hypothesized (Stanica et al 2008; Stavrakoudis et al. 2001) that the RAD fragment of the octapeptide, which is homologous of the well known RGD sequence,
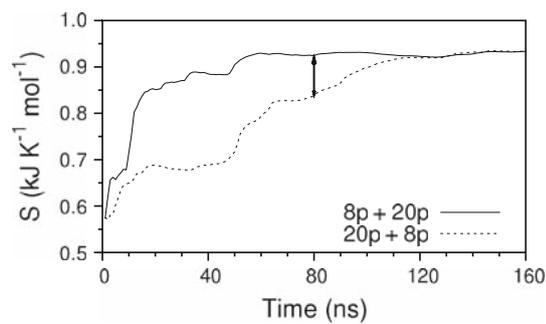
Fig. 6 Plot of estimated entropies of backbone atoms of the 314-319 fragment of the P1 and P2 peptides. Frames from the P1 were appended to the P2 trajectory and vise-versa. Last 80 ns from the two trajectories were used, assuming equilibrium was reach. See "Methods" for details of calculation. *Arrow* at 80ns indicates the entropy difference in conformational entropy of the backbone atoms

Table 3 Hydrogen interactions found in the P2 (20mer) peptide trajectory

Donor	Acceptor	% Occurrence
$Tyr_{313}:N$	$Glu_{324}:O^{\varepsilon 1,2}$	14.7
$Met_{314}:N$	$Leu_{322}:O$	36.9
$Glu_{315}:N$	$Leu_{322}:O$	5.2
$Glu_{315}:N$	$Ala_{323}:O$	
$Arg_{317}:N$	$Glu_{315}:O$	20.5
$Arg_{317}:N$	$Arg_{320}:O$	17.3
$Arg_{317}:N^{\varepsilon}$	$Glu_{315}:O^{\varepsilon 1,2}$	35.4
$Arg_{317}:N^{\eta 2}$	$Glu_{315}:O^{\varepsilon 1,2}$	43.0
$Leu_{322}:N$	$Glu_{315}:O$	37.4
$Val_{325}:N$	$Tyr_{313}:O$	15.3
$Arg_{327}:N^{\varepsilon}$	$Glu_{324}:O^{\varepsilon 1,2}$	24.6
$Arg_{317}:N^{\eta 1,2}$	$Glu_{324}:O^{\varepsilon 1,2}$	29.0

plays a significant role in the biological activity of the $Y_{313}MESRADR_{320}$. The side chain orientation of the arginine and aspartic residues has been classified as an important factor to inhibitory activity of such peptide analogues (Kostidis et al 2004; Stavrakoudis and Tsikaris 2008; Stavrakoudis et al. 2000). As a consequence, a dihedral angle of orientation (*pdo*) has been introduced, in order to account this particular conformational feature. The four atoms that constitute this dihedral angle are $Arg_i:C^{\zeta}$, $Arg_i:C^{\alpha}$, $Asp_{i+2}:C^{\alpha}$ and $Asp_{i+2}:C^{\gamma}$ atoms, where $i =317$ in one case for example (Arg_{317}-Asp_{319}). As it shown in Fig. 7, the P2 peptide preferred *pdo* values around −30° for the Arg_{317}-Ala-Asp_{319} fragment. At the same time, this pdo angle showed wider distribution in the P1 peptide, with values closer to −90°. This is somewhat expected, if we take into consideration the previously discussed unordered structure of the P1 peptide. Similar preference, although
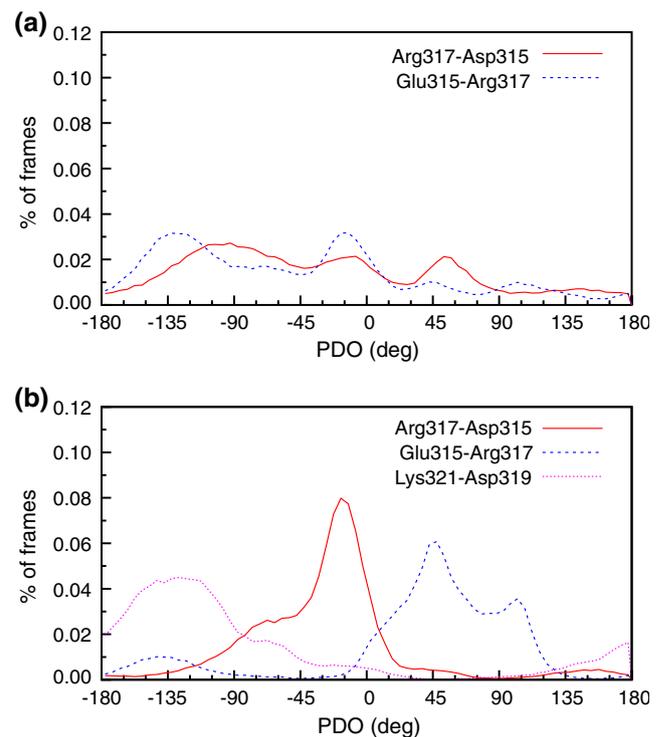

Fig. 7 Probability distribution of the pdo dihedral angle of orientation of the Arg/Lys and Asp side chains in **a** P and **b** P2 trajectories, respectively. Probabilities were calculated within 5° angle bin

with opposite signs ($\sim 90°$) has been observed in Ac-RCDC-NH2 peptide (Stavrakoudis and Tsikaris 2008). The corresponding value of *pdo* in the X-ray structure (starting conformation) was found 11.6°. Analysis of the NMR obtained bundle of structures of Ac-RGD-NH$_2$ peptide in DMSO solution (Biris et al 2003b) revealed that *pdo* dihedral angle averaged at −54.7° (17.6°) and ranged between −15.4° and 70.9°. These facts indicate that experimental results obtained from NMR or X-ray experiments and simulation results presented in this work advocate in a relative synplanar orientation of polar side chains of arginine and aspartic acid residues in RXD sequence. Proximity of these opposite charged groups is somewhat expected, although distances between $Arg:C^{\zeta}$ and $Asp:C^{\gamma}$ atoms were found above 0.6 nm (can be 1.5 nm in some cases) indicating side chains can maintain synplanar orientation despite the weak nature of electrostatic interactions between them. Recent experimental and theoretical studies of model RGD peptides in gas phase also provided evidence for the synplaner orientation of Arg and Asp side chains (Li et al 2009; Gregoire et al 2007).

Both P1 and P2 peptides exhibited similar biological activity (Biris et al 2003b), despite the substantial differences in their conformation. This fact implies that the relatively high backbone flexibility of the P1 (8mer) peptide did not result in significant loss of activity.

Conclusions

The peptide fragments derived from the α_{IIb} sequence have studied with molecular dynamics simulations and their conformation has been analyzed. Sequences of 313-320 (P1) and 313-332 (P2) of the α_{IIb} protein, component of the integrin receptor, have been identified using epitope mapping techniques of overlapping peptides, to play important role in fibrinogen binding and platelet aggregation. The current study presented conformational analysis of these peptides in aqueous solution, by utilizing computer modeling and molecular dynamics simulations.

Both peptides showed considerable conformational flexibility. The P2 (20mer) analogue retained partly its initial strand conformation but the P1 analogue lost completely its initial conformation during MD trajectory. Despite this flexibility P1 did not lost its biological activity. As it has been hypothesized elsewhere (Mitsios et al. 2006; Papamichael et al. 2009; Stanica et al. 2008; Stavrakoudis et al. 2000), peptide with 313-320 sequence retained the ability to inhibit the platelet aggregation and fibrinogen binding, through the presence and side chain specific orientation of the RAD residues. The current study provides evidence of this synplanar orientation of Arg/Asp side chains, commonly found in both P1 and P2 analogues, despite the differences in backbone conformational status. The results are in line with previously reported analyses of NMR and X-ray data on similar sequences. This study provides also evidence that biologically active peptides, does not always have a well defined structure.

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