Report 2 - Simulation of S-peptide

Alicja Nowakowska, 234857

May 2020

1 Introduction

This report aims to simulate the S-peptide of ribonuclease A using molecular dynamics technique. S-peptide is a short peptide that consists of 20 amino acid residues in the following order: KETAAAKFERQHMDSSTSA [13] and along with S-protein is the enzymatically inactive product of the digestion of ribonuclease A by subtilisin . It is responsible for binding the S-protein to form the enzymatically active ribonuclease S [8]. The S-peptide's structure was widely studied and it is known that 4-12 residues form the α-helix whose stability was investigated experimentally and it depends on [2]:

- temperature - when the temperature increases the α-helix contribution decreases. It indicates that enthalpy impacts on the helix formation. The unusual fact that the significant α - helix formation exists in water at 0° for S-peptide attracted the attention of the scientific community.
- pH - the pH dependent interactions: one between the aromatic rings $\mathrm{Phe}^{8} - \mathrm{His}^{12+}$ and the side chain one between the residues $\mathrm{Glu}^{2-} - \mathrm{Arg}^{10+}$ are the main source of the helix stability. Glu^{2} and Arg^{10} form a salt bridge.
- the complementary electrostatic interactions between charged groups located close to the C- and N-termini
- helix dipole
- protonation of His^{12}.

The rest of the residues forms a random coil. Moreover, it was examined that the residue His^{12} is responsible for the enzymatic catalysis and Met^{13} and Phe^{8} function as scaffolding. Frequently, the analogues of the S-peptide were studied in order to identify individual residue's properties. It was proven that the removal of Lys^{1} has no significant impact on activity and dissociation while the removal of Glu^{2} increases dissociation by a factor of 4. Phe^{8}, Met^{13} and His^{12} were identified to stabilize the transition rate [9].

1.1 Molecular dynamics investigation of the S-peptide

The conformational changes and folding-unfolding mechanisms are the main topics of the peptide investigation in molecular dynamics. The molecular dynamics studies shall enable the understanding of the conformational preferences of the amino acids and gaining insights into the mechanism of helix formation, stabilization, disruption thanks to the analysis of the system time evolution.

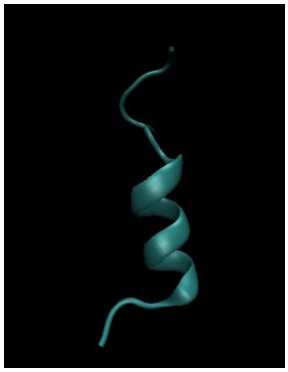
The main obstacle that arises when dealing with this kind of simulation is the time scale. To observe a biologically significant event such as protein folding the integration time step of the order of femtoseconds and the simulation time of microseconds is needed. The conventional simulations frequently do not permit the observation of the interesting events such as for example mechanisms of transitions between different conformational states since the system tends to stuck to the locally stable states and only the local fluctuations may be observed [12]. Nevertheless, thanks to the recent advances, nowadays it's possible to explore the folding of small proteins (¡80 amino acids) and the conformational space of peptides. It was proven that still the further research in the adjustment of the proper force field and the adequate conformational sampling is essential [6]. The effect of the solvent was also investigated and its correct treatment turned out to be crucial for the realistic simulation of peptide folding. The 3 main approaches for the solvent treatment were developed: ignoring its effect, creation of an external field acting on the peptide or protein and the explicit inclusion. The last one proven to be the best [10].

In [11] the molecular dynamics simulations of the S-peptide revealed the pressure dependence of the conformation clusters, energy and density. The used model was GROMOS96 for the peptide and SPC for the water. The considered temperature was 298 K and pressure 1 atm. Within few picosecends the pressure and density reached equilibrium and total system was considered completely equilibrated after 10 ps. The RMSD oscillated around 0.14 nm, total energy around -12000 $\frac{\text{kcal}}{\text{mol}}$ and density around 900 $\frac{\text{kg}}{\text{m}^3}$. The obtained results were consistent with the experimental ones. In [5] the simulations of the S-peptide shown that it maintains its stable helical native structure at 278 K while it unfolds completely in 20 nanoseconds at high temperatures (358 K). In another S-peptide simulation study[7] the helix turned out to be stable for 300 picoseconds at 278 K and unfolded in less than 500 picoseconds at 348 K though clearly the equilibrium was not reached. In [9] it was demonstrated that all-atom MD simulations can predict structures of cyclic peptides and other peptide-based foldamers with accuracy similar to experiments and the significance for complex stability of Arg^{10} was shown. The used methodology included the AMBER type and TIP3P force fields. Furthermore, in this article the S-pepitde's helicity was studied. The extensive md of 10 ms at 298 K was performed with the starting point of the initial helical conformation. During the simulation S-peptide turned out to go through a continuous refolding process. The most of the helical content was confined to residues from Lys^7 to Arg^{10} - the smallest regions required to restore RNase-S enzymatic activity. The salt bridge between Glu^2 and Arg^{10} repeatedly opened and closed during the simulation. Some estimation errors were also observed probably due to the inaccuracy of the used force field. In [4] a new MD method was presented on the S-peptide example - Directed Essential Dynamics. The results indicated that DED is capable of speeding up the peptide folding procedure. Also the traditional MD was performed for the comparison and its tendency to stick to the local minima was confirmed. The simulation time was 20 ns, pressure 1 atm, temperature 298 K and the force field - Charmm27. The RMSD oscillated all the time around 0.1 nm and the radius of gyration around 0.6 nm.

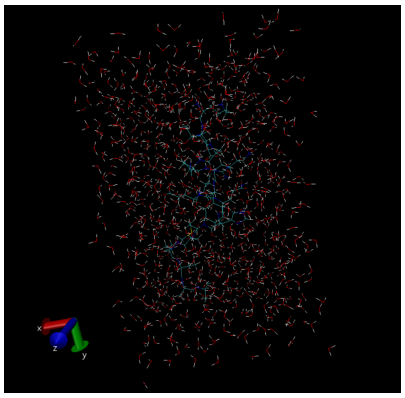
2 Methodology

The simulation in this report is performed using GROMACS software. The first step is the setting of the initial simulation parameters. In the considered case the temperature is equal 300 K, the reference pressure for coupling is 1 bar, the number of water molecules is 871 and the the considered peptide length is 19 residues. It's assumed that the molecules' configuration is limited to the certain box space. The initial configuration of the system is shown in the Figure 1. The length of the

simulation is 100 ps with the time step 0.002. In the next steps atoms of the water move according to TIP3P type force field and atoms of the peptide according to the AMBER type force field. In each iteration all the atoms are moved and the system parameters are stored. In this way it's possible to observe and analyse their time evolution.



(a) Protein



(b) Protein in water

Figure 1. Visualization of the initial state of the system

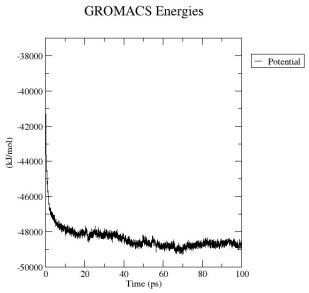
The simulation performance consisted of 3 parts. First the minimization energy was done, then the simulation with restraints and then without them was performed.

3 Convergence check

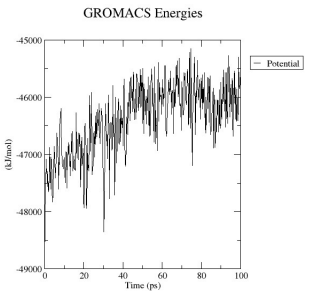
Before analysing the results the convergence of the system for both simulations - with and without restraints must be checked what is shown in the Figure 2. It may be observed that the simulation with restraints enables speeding up the convergence of the system. Just after 20 ps the limit seems to be reached and the fluctuations are rather small. When it's finished the system state is preserved and the simulation without restraints is performed. Apparently freeing the atoms cause significantly higher fluctuations and the equilibrium for all the considered properties seems to be reached in much longer time - around 60 ps. However, the stability is not completely certain and shall be treated with caution.

Interestingly, the plots for the Protein-Solvent are the most similar and stable ones.

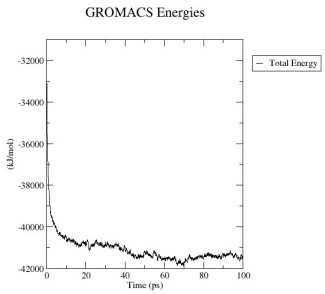
Moreover, it is worth to stress that if the simulation with restraints was not previously performed without no doubt the equilibrium reaching would take longer.



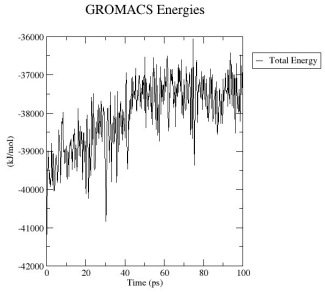
(a) MD with restraints



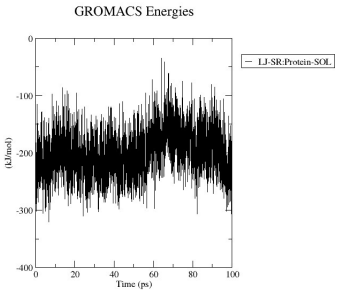
(b) MD without restraints



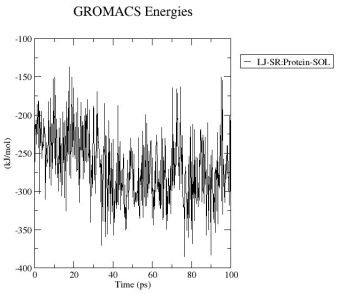
(c) MD with restraints



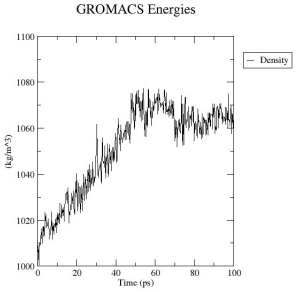
(d) MD without restraints



(e) MD with restraints



(f) MD without restraints



(g) MD without restraints

Figure 2. Convergence check

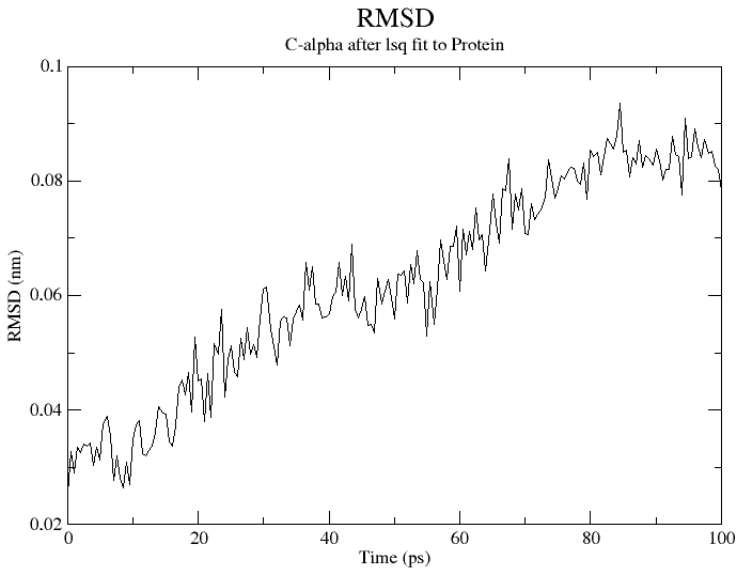


Figure 3. RMSD

4 Analysis

In this section the S-peptide simulations results are analysed.

4.1 RMSD

The Root Mean Square Displacement is the measure of the distance between the initial position of the structure and the current one. The plot is presented in the Figure 3. Frequently, it's used by scientists to diagnose the equilibrium reaching. In the considered case the RMSD seems to be constantly raising with the small local stability moments. Such period is apparent between 80-100 ps of the simulation and 40-60 ps. It's possible that after 80 ps the equilibrium is reached though having in mind the conclusion from [3] - RMSD is not the best measure for the equilibrium - this diagnosis should be treated with caution.

The RMSD values are at the same time rather small what indicates that only the slight changes appear in the peptide structure.

4.2 Radius of gyration

The next step in the analysis is the Radius of Gyration plot that measures how compact the protein is. When it raises it unfolds and when decreases it gets closed-packed. It is shown in the Figure 4. The taken value oscillates around 0.5 nm.

It may be concluded that the peptide's compactness is stable in time. No significant changes are observed.

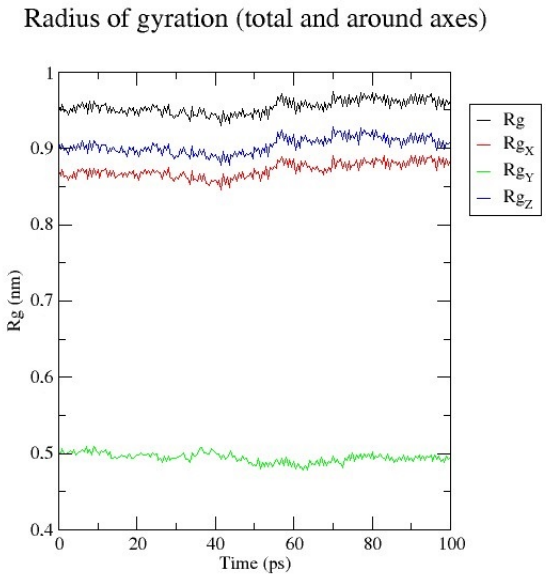


Figure 4. Radius of gyration

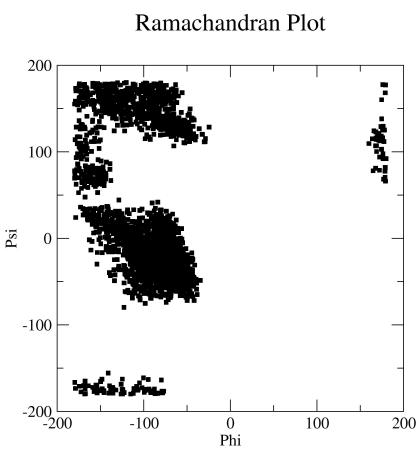
4.3 Ramachandran plot

In the Figure 5 the Ramachandran plot and its interpretation graph are presented. This part of the analysis is performed in order to check if the used model predicts correctly the angles and the corresponding structure. If the points are out of the allowed region model predicts the structure that does not exist in nature. In the considered case all points suit the scheme. Nevertheless, the structure angles indicate some beta helix formation what is inconsistent with the true S-peptide construction. Thus, the used simulation model is not completely adequate.

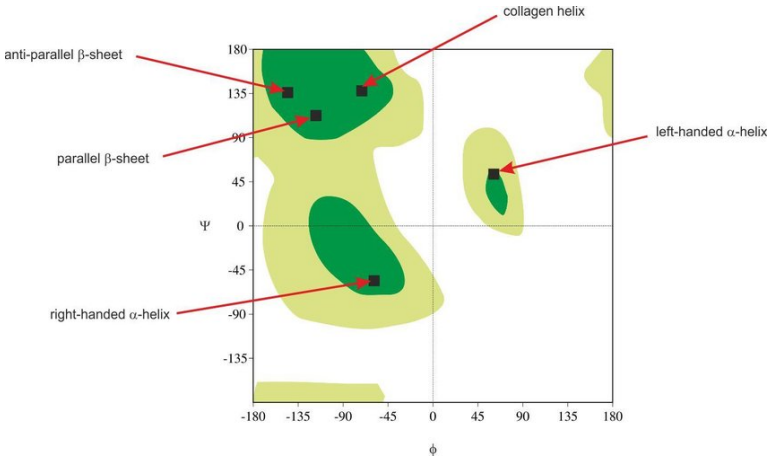
4.4 Salt bridges

The salt bridge is defined to exist if the distance between any of the oxygen atoms of acidic residues and the nitrogen atoms of basic residues are within the cut-off distance (default 3.2 Angstroms) in at least one frame. It represents an interaction between two groups of opposite charge in which at least one pair of heavy atoms is within hydrogen bonding distance. It is a combination of hydrogen bonding and electrostatic interlinkage. The salt bridge is known to have impact on the helix stability, molecular recognition and catalysis usually involving charged amino acids like Asp or Glu (negative) and His, Arg, or Lys (positive).

Using VMD it was diagnosed that the salt bridge exist in the simulation for the S-peptide between the Glu and Arg residues. In the Figure 6 the distance between the four pairs of Oxygen-Nitrogen atoms of the residues is shown in time. The criterion of 3.2 Angstroms is always fulfilled for one of the Nitrogen-Oxygen pair (the first and second have the strongest interaction) thus the salt bridge is always present. The third and the fourth pair of the atoms forms some kind of quasi salt bridge - the distance between them marks fluctuations and the salt bridge is formed and broken



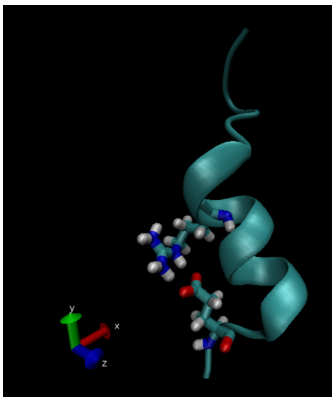
(a) Ramachandran plot



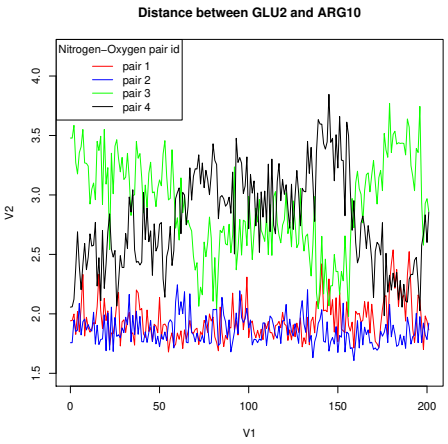
(b) Ramachandran plot interpretation [1]

Figure 5. Ramachandran plot

few times.



(a) Dependence of the distance between residues on time



(b) Salt bridge visualization

Figure 6. Salt bridge analysis

4.5 Secondary structure analysis

In the Figure 7 the secondary structure in time is presented (pink region corresponds to alpha helix, white to random coil and green to turn). It may be noted that the essential alpha-helix formation between the Lys and Arg residues is always preserved. Generally, it may be said that the alpha helix

is preserved due to the constant presence of the salt bridge between Arg and Glu that guarantee its stability. Moreover, it may be noticed that the turn part disappears the longer the simulation is, what indicates that less and less changes appear in the system, finally reaching the true structure in the last iterations.

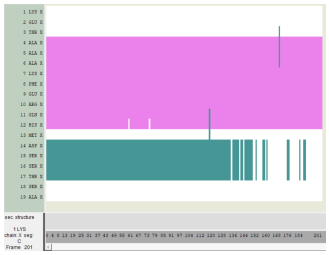


Figure 7. Secondary structure in time

5 Summary and discussion

To sum up, in this report the simulation of the S-peptide using molecular dynamics was performed. In the Table 1 the obtained results and the corresponding ones from other papers are summarized.

Table 1. Comparison of the results

Property	Simulation value	Reference simulation value at 298 K and 1 bar
total energy	oscillation around $-40000\frac{\text{kJ}}{\text{mol}}$ $(9553\frac{\text{kcal}}{\text{mol}})$	oscillation around $-12000\frac{\text{kcal}}{\text{mol}}$ [5]
RMSD	oscillation around 0.08 nm	oscillation around 0.14 nm [5]; oscillation around 0.1 nm [4]
Radius of gyration	oscillation around 0.5 nm	oscillation around 0.6 nm [4]
Secondary structure	alpha helix is stable	alpha helix is stable [5] [7]; some refolding process may be observed [9]

It may be noticed that the values are quite similar. Probably some minor discrepancies appear due to the different force field approach or different software. Moreover, as it was predicted in the Introduction section the short molecular dynamics simulation as it happens in the considered case, tends to stick to the local minima. The alpha helix is completely stable. Also the radius of gyration seems not to change in time at all. Nevertheless, the RMSD plot revealed that the peptide does experience some slight metamorphosis and that the system configuration is not equal to the

initital one though the changes are almost innotable and insginificant. Other MD simulations of the S-peptide of the similar length revealed similar properties though more extensive MD studies (simulations of microseconds) shown other behaviour of the peptide indicating the artifacts in the force fields. In the considered case the S-peptide behaves in the desired way consistent with the experimental results. The salt bridge appearance was noted influencing the helix stability and indicating which pair of the atoms has the strongest electrostatic interaction.

This study confirmed that the short MD S-peptide simulation enables the investigation of its properties and behaviour.

References

[1] Jad Abbass. *Secondary Structure-Based Template Selection for Fragment-Assembly Protein Structure Prediction*. PhD thesis, Kingston University London, 2018.

[2] Kurt D. Berndt Ana Caballero-Herrera, Kerstin Nordstrand and Lennart Nilsson. Effect of urea on peptide conformation in water: Molecular dynamics and experimental characterization. *Biophysics Journal*, 89:842–857, 2005.

[3] M. Cibena W. Schreiner B. Knapp, S. Frantal and P. Bauer. Is an intuitive convergence definition of molecular dynamics simulations solely based on the root mean square deviation possible? *Journal of Computational Biology*, 18:997–1005, 2011.

[4] Linsen Zhang Changjun Chen, Yi Xiao. A directed essential dynamics simulation of peptide folding. *Biophysical Journal*, 88:3276–3285, 2005.

[5] Jianbin He Yunyu Shi Haiyan Liu, Zhiyong Zhang. *Normal Mode Analysis: Theory and Applications to Biological and Chemical Systems*, chapter Using collective coordinates to guide conformational sampling in atomic simulations. Chapman and Hall/CRC, 1 edition, 2005.

[6] Jing Ye Hao Geng, Fangfang Chen and Fan Jiang. Applications of molecular dynamics simulation in structure prediction of peptides and proteins. *Computational and Structural Biotechnology Journal*, 17:1162–1170, 2019.

[7] Scott M. LeGrand Kenneth M.Jr. Merz. *The Protein Folding Problem and Tertiary Structure Prediction*, chapter Molecular Dynamics Studies of Folding and Unfloding. Birkhauser, 1994.

[8] Raines RT Kim JS. Ribonuclease s-peptide as a carrier in fusion proteins. *Protein Science*, 2:348–356, 1993.

[9] Rainer Bomblies Manuel P. Luitz and Martin Zacharias1. Comparative molecular dynamics analysis of rnase-s complex formatio. *Biophysical Journal*, 113:1466–1474.

[10] Wilfred F. van Gunsteren Xavier Daura, Alan E. Mark. Peptide folding simulations: no solvent required? *Computer Physics Communication*, 123:97–102, 1999.

[11] Takahisa Yamato. *High Pressure Bioscience and Technology*, volume 13, chapter Finite element study of protein structure under high pressure. Elsevier Science, 1 edition, 1996.

[12] Yunyu Shi Zhiyong Zhang and Haiyan Liu. Molecular dynamics simulations of peptides and proteins with amplified collective motions. *Biophysics Journal*, 84:3583–3593, 2003.

[13] Yunyu Shi Zhiyong Zhang, Yongjin Zhu. Molecular dynamics simulation of urea and thermal-induced denaturation of s-peptide analogue. *Biophysical Chemistry*, 89:145–162, 2001.