



ISSN 1823-626X

Journal of Fundamental Sciences

available online at <http://jfs.ibnusina.utm.my>



Molecular Dynamics Folding Simulation of β -hairpin Protein (1E0Q)

Nur Shima Fadhilah Mazlan¹ and Nurul Bahiyah Ahmad Khairudin²

¹Faculty of Chemical Engineering, Universiti Teknologi Malaysia Johor Bahru, 81300, Johor Malaysia

²Malaysia Japan International Institute of Technology, Universiti Teknologi Malaysia International Campus, Jalan Semarak, 54100, Kuala Lumpur Malaysia

Received 1 September 2011, Revised 25 November 2011, Accepted 30 November 2011, Available online 10 December 2011

ABSTRACT

The structure and trajectories of the mutant peptide of ubiquitin (PDB ID: 1E0Q) has been studied using Molecular Dynamics (MD) simulation. The simulation was performed using AMBER 11 utilizing force field 99 for 50 ns at constant temperature 325 K. The purpose of this study is to investigate the protein folding pathway of protein 1E0Q. In this simulation, the protein 1E0Q has folded into its near native β -hairpin structure within 5 ns. The RMSD value as compared to the NMR structure from the first residue to 17 residues is 2.17 Å. It has been observed that Gly 10 had been responsible to promote β -turn which caused the structure to turn into β -hairpin. In secondary structure analysis, it is shown that the residue from Thr 6 to Lys 11 has formed a bend in the structure. Two beta strands has also been found comprising residues Glu 2 to Lys 5 and Ile 13 to Glu 16.

| Molecular Dynamics | Protein Folding | β -hairpin | Molecular Simulation |

© 2011 Ibnu Sina Institute. All rights reserved.
<http://dx.doi.org/10.11113/mjfas.v7n2.257>

1. INTRODUCTION

Protein folding is the physical process where the polypeptide folds into a unique functioning three-dimensional (3D) structure. Lately, various proteins folding pathway has been investigated by experimentally and theoretically [1] and it had drawn many interested hypothesis but still had a long journey in understanding the mechanism of protein folding. Experimental study such as NMR Spectroscopy and X-ray Crystallography provides the native protein structure of the protein [2] but it is failed to explain the protein folding pathway due to the ultra fast protein folding speed [3]. The smaller protein can folded in less than 1 s, while the bigger protein can completely folded up to a few minutes time.

It has been known that, of all the molecules found in living organisms, proteins are the most important. They are used to support the skeleton, control senses, move muscles, digest food, and defend against infections and many other functions. But, how exactly for a protein fold from linear chain into its native 3D structure yet still become a mystery to be solved. In fact, the folding process of proteins had been stated as one of the biggest unsolved problems in science [4]. The more complicated is that the proteins can be fold and refold and sometimes it can misfolded [5]. It is the world worries because the incorrect protein folding has been cause to some disease such as Alzheimer and Mad Cow Diseases [6].

Thus, it is important to study and understand the responsible interaction in the protein that makes the protein folded correctly. The protein folding process can be investigate using MD simulation, which is had been proposed by many researcher to be the best method on describing the protein at atomic level detail [7].

However, due to the complexity of the protein folding, MD Simulation can only be solved up to micro second (μ s) only and it also needs a supercomputer to the simulation [8]. The simulation processes on a long timescales (beyond 1 μ s) are really expensive because it requires so many time steps. Thus, it is easier to pick the smaller protein to gain more understanding for protein folding pathway. Smaller protein such as β -hairpin posses many characteristic of proteins in their behavior and also it's their two site transition that had proved in the experimental [9].

A β -hairpin is a small protein structure motif in which having two β -strands, linked by a turn fold to form hydrogen bonds with each other. It is the simplest model for protein study and it had been proposed as an initiation sites in early protein folding event [10]. Thus, understanding the mechanism of formation of β -hairpin can provide useful information for the folding of larger proteins. It has been aim for this study to investigate the mechanism of the early stage on protein folding for further understanding in formation of β -hairpin protein.

Corresponding author at: Malaysia Japan International Institute of Technology,
Universiti Teknologi Malaysia International Campus,
Jalan Semarak, 54100, Kuala Lumpur Malaysia
E-mail addresses: nurulbahiyah@ic.utm.my (Nurul Bahiyah Ahmad Khairudin)

2. EXPERIMENTAL

2.1 Materials and Method

This study focuses on simulating a small protein or peptide which consist less than 20 residues in molecular dynamic. Mutant peptide of ubiquitin (PDB code: 1E0Q) had been selected from Protein Data Bank (PDB) to run this simulation. This protein is registered in the PDB's profile having β -hairpin structure [11]. The sequence that be used in this simulation is from first terminal to 17 amino acid of ubiquitin. The acid amino sequence is NH₂-Met-Gln-Ile-Phe-Lys-Thr-Leu-Asp-Gly-Lys-Thr-Ile-Thr-Leu-Glu-Val-COOH.

Protein 1E0Q was simulated using an all atom classical simulation and slightly modified version of AMBER 11[12] force field 99[13]. The energy minimization was carried out using 500 cycles of steepest descent and another 500 cycles of conjugate gradient [14]. The MD simulation was carried out for 50ns. The simulations were performed in a periodic boundary condition [15] with the molecule immersed in a truncated octahedron water box filled with TIP3P water model [16]. Non-bonded interactions were truncated by using a 12 Å cutoffs and PME, for Lennard Jones and coulumbic interactions respectively. The system was coupled to a temperature bath using Berendsen [17] thermostat to maintain the temperature at 325K with coupling constant of 1.0 picoseconds (ps).

Bond constraints were imposed on all bonds involving hydrogen atoms via SHAKE [18]. The trajectories were produced by numerical integration of the Newton's equation of motion using the Verlet Algorithm [19] with a time step of 2 femtosecond (fs).

3. RESULTS & DISCUSSION

3.1 Root Mean Square Deviation (RMSD)

All RMSD calculation was performed in order to make a quantitative comparison between the simulation structure and the native structure. The RMSD value to the

NMR structure had been plotted versus the simulation time (Figure 1.0). The RMSD was initially constant and then decreased to a value of around 4.5 Å at 4.5 ns to 5.5 ns. Then it increased up to 5 Å. It shows that the MD Simulation structure has the most similarity with NMR structure within 4.5 ns to 5.5 ns.

To accelerate the conformational folded structure, the temperature of the MD system is set to be slightly higher than the experimental study [11] which is 300 K. This is the strategy that had been brought by Bonvin and van Gunsteren [20] which had performed MD simulation for a 19-residue peptide from α -amylase inhibitor tendamistat at high temperature 360K. By setting the temperature of MD simulation at 325K, the folded protein 1E0Q had been obtained at 5 ns which have the lowest RMSD value, 2.17 Å (Refer Figure 1.0).

Although this result is not good as Jang et al [21] which obtained 1.36 Å, it is still acceptable result. This is due to some reasonable reason. The result is different from other finding due to the length of simulations on different computer and/or different numbers of processor and memory. For this simulation, a computer with processor Intel i3 Core 2 Duo with software AMBER version 11 was used.

Other reason is due to the way molecular dynamics works, small variations in the order of execution and rounding in the floating point calculations. Its mean that the trajectories sampled by different machines will diverged over time. The result obtained in this simulation is not an error or bugs cause neither one simulation more correct than another. It is simply that the two simulations are exploring different regions of phase space. The result could be possibly the same if using the same parameter with the same specification machine or computer.

While, the protein folding pathway of protein 1E0Q can be observed through a series of snapshot starting from 0 ns to 10 ns as shown in Figure 3.0. The dash lines colored by residue represent the hydrogen bond. As it can be seen, the hydrogen keep forming and reforming to stabilize the structure. The analysis of hydrogen bonding was further carried out in order to study the protein interaction.

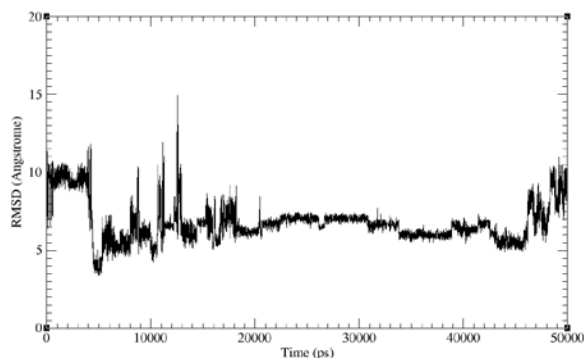


Fig. 1 Graph of RMSD to NMR Structure versus Time

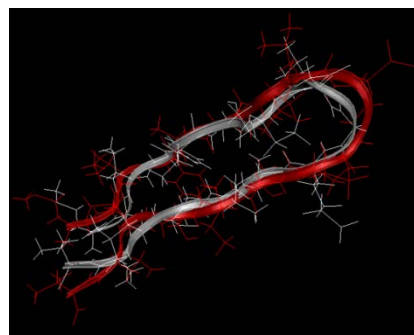


Fig. 2 Superimpose of model (White) to native NMR Structure (Red)

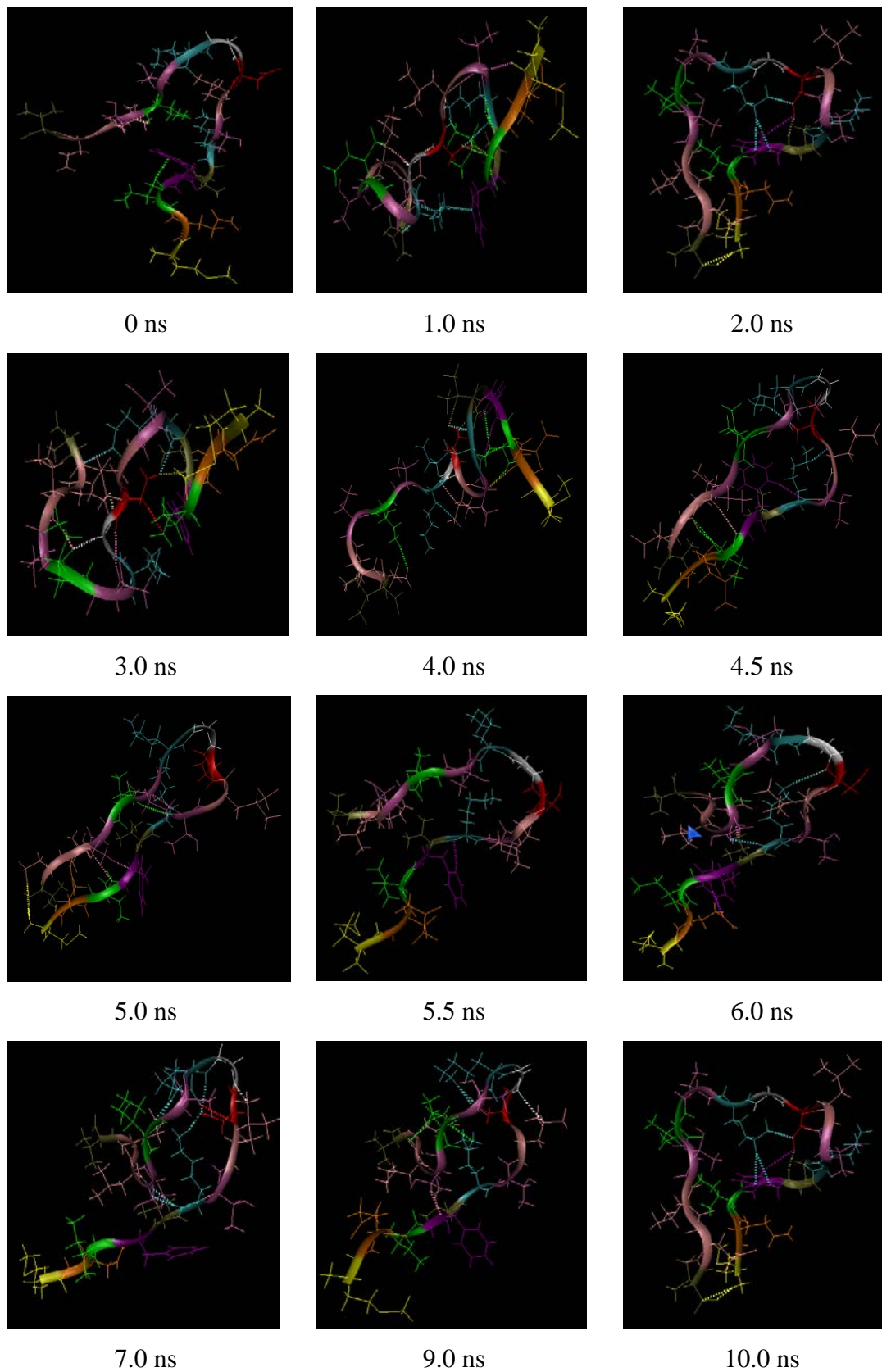


Fig. 3 Folding pathways of protein 1E0Q for simulation time of 10 ns

3.2 Hydrogen Bonding

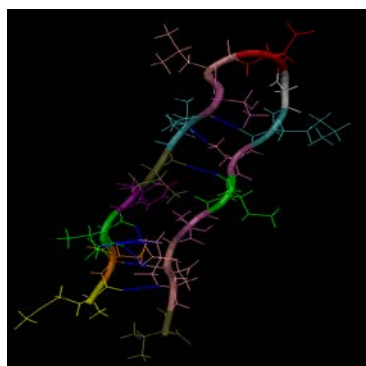


Fig. 4(a) NMR native with hydrogen bonds shown in blue line.

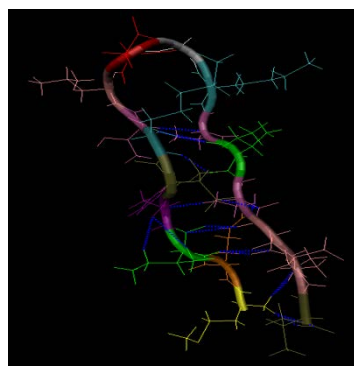


Fig. 4(b) Model structure with hydrogen bonds shown in blue line.

Table 1 Comparison of stable backbone hydrogen bonds between NMR Structure and the model structure

Donor (N) – Acceptor (O)	NMR Structure (Å)	MD Simulation Structure (Å)
VAL 17 (N) – MET 1 (O)	3.70	2.92
LEU 15 (N) – ILE 3 (O)	3.06	2.90
GLY 10 (N) – LEU 8 (O)	3.60	2.87
ILE 3 (N) – LEU 15 (O)	3.30	2.84
MET 1 (N) – GLU 16 (O)	3.60	2.87
MET 1 (N) – VAL 17 (O)	3.70	2.91

One of the major factors that contribute in the correct folding of proteins is the formation of hydrogen bonds. A hydrogen bond occurs when two electronegative atoms, such as nitrogen and oxygen, interact with the same hydrogen. The hydrogen is normally covalently attached to one atom, the donor but interacts electrostatically with the other, the acceptor. This interaction is due to the dipole between the electronegative atoms and the proton.

In this analysis, hydrogen bond is defined by the distance between an oxygen atom and a polar hydrogen atom with range shorter 3.6 Å. As shown in Figure 4(a) and 4(b), there was a minor difference in the number of hydrogen bonds for both structures. The hydrogen bonds are representing by blue dashes while the protein structure is colored by residue name. It seemed that the model had more numbers of hydrogen bonds compared that of the native.

For the better proved data, the length of stable backbone hydrogen bonds between NMR structure and MD simulation structure had been compared and tabulated in Table 1.0. Note that, the Table 1.0 is only comprised the hydrogen bond data that can be compared with each other. From the data shown, it is clearly seen the different distance value in term of hydrogen bond contact between NMR structure and MD Simulation structure.

3.3 Secondary Structure Analysis

The secondary structure analysis had been done using PolyView 2D [22], to validate and conformed the protein model structure.

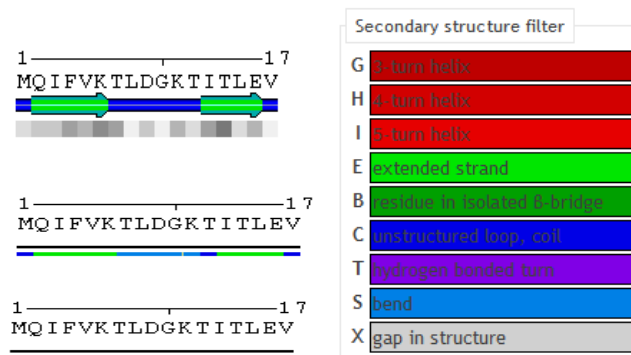


Fig. 5 Result of Secondary Structure Analysis and Legend for Secondary Filter

From the Figure 5.0, it shown extended strand which is beta strand in this simulation from Glu 2 to Lys 5 and from Ile 13 to Glu 16. While, a bend was found at residue between Thr 6 to Lys 11 and there is also a coil founded which is unstructured loop at Met 1, Thr 12 and Val 17.

By looking at the series of snapshot of protein structure in Figure 3.0, it is clear that establishment of a turn in the middle of the chain is the key event leading to the formation of the β -hairpin. From the POLYVIEW 2D result, it is found that the Gly 10 responsible to promotes the β -turn that make the protein structure turn into β -hairpin. In this MD simulation, No α -helical structure was encountered and there was no persistent helicity observed for any individual residues, in agreement with the NMR study [11].

4. CONCLUSION

In this study, the protein 1E0Q had folded into its near native β -hairpin structure within 5 ns. The RMSD value as compared to the native structure was found to be 2.17 Å. Through observation on the folding pathway, it can be suggested that Gly 10 was responsible to promote β -turn which causes the protein structure to form β -hairpin.

ACKNOWLEDGEMENTS

The authors would like to thank Universiti Teknologi Malaysia (UTM) Johor Bahru for technical support as well as MOSTI and IFN Grant for funding this project.

REFERENCES

- [1] O.V. Galzitskaya, J. Higo and A. V. Finkelstein, Alpha-helix and beta hairpin folding from experiment, analytical theory and molecular dynamic simulation, *Curr. Protein Pept. Sci.* 3 (2002) 191-200.
- [2] R.A. Friesner and J. R. Gunn, *Computer Simulation of Protein Folding*, Computational Studies of Protein Folding, 2003.
- [3] J. Kubelka, W. A. Eaton and J. Hofrichter, Experimental test of villin subdomain folding simulations, *J. Mol. Biol.* 329 (2004) 625-630.
- [4] Editorial: So much more to know, *Science* 309 (2005) 78-102.
- [5] V. Pande and D.S. Rokhsar, Molecular dynamics simulation of unfolding and peptide revealed from molecular dynamics simulation in explicit water, *Biophysical Journal* 86 (1999) 1946-1968.
- [6] W.A. Thomasson, *Unravelling the Mystery of Protein Solving* (2005)
- [7] V. Dagget, *Protein Folding – Simulation*, *Chemistry Review* 106 (2006) 1898-1916.
- [8] K.A. Dill, S.B. Ozkan, R.W. Thomas, J.D. Chodera and V.A. Voelz, The protein folding problem : when will it be solved?, *Current Opinion in Structural Biology* 17 (2007) 342-346.
- [9] R.J. Munoz-Gotera, E.O. Hernandez-Gonzalez, G. Mendoza-Hernandez, R.G. Contreras and A. Mujica, Exocytosis of a 60 kDa Protein (Calreticulin) From Activated Hamster Oocytes, *Molecular Reproduction and Development* 60 (2001) 405-413.
- [10] S. G. Chang et al, *Bull. Korean Chem. So.* 23 (2000) 1369.
- [11] R. Zerella, P.Y. Cheng, P.A. Evans, A. Rainee and D.H. Williams, Structural characterization of a mutant peptide derived from ubiquitin : Implications for protein folding, *Protein Science* 9 (2000) 2142-2150.
- [12] D.A. Case, T.A. Darden, T.E. Cheatham III, C.L. Simmerling, J. Wang, R.E. Duke, R. Luo, R.C. Walker, W. Zhang, K.M. Merz, B. Roberts, B. Wang, S. Hayik, A. Roitberg, G. Seabra, I. Kolossvary, K.F. Wong, F. Paesani, J. Vanicek, J. Liu, X. Wu, S.R. Brozell, T. Steinbrecher, H. Gohlke, Q. Cai, X. Ye, J. Wang, M.-J. Hsieh, G. Cui, D.R. Roe, D.H. Mathews, M.G. Seetin, C. Sagui, V. Babin, T. Luchko, S. Gusarov, A. Kovalenko, and P.A. Kollman, *Amber 11*, University of California, San Francisco, 2010.
- [13] D.A. Case, T. Cheatham, T. Darden, H. Gohlke, R. Luo, K.M. Merz, Jr., A. Onufriev, C. Simmerling, B. Wang and R. Woods, The Amber biomolecular simulations programs, *J. Computat. Chem.* 26 (2005) 1668-1688.
- [14] A. R. Leach, *Molecular Modelling: Principles and Applications*, Second edition, Pearson Education EMA, 2001
- [15] R.C. Walker, M.F. Crowley, D.A. Case, The implementation of a fast and efficient hybrid QM/MM potential method within the Amber 9.0 with sander module, *J. Computat. Chem* 29 (2008) 1019-1031.
- [16] W.L. Jorgensen, J. Chandrasekhar, J.D. Madura, and R.W. Impey, Comparison of simple potential functions for simulating liquid water, *J. Chem. Phys* 79 (1983) 1407-1413.
- [17] H.J.C. Berendsen, J.P.M. Postma, W.F. Van Gunsteren, A. DiNola, J.R.J. Haak, *Chem. Phys.* 81 (1984) 3684-3690.
- [18] J.P. Ryckpaert, G. Ciccotti, and H.J.C. Berendsen, Numerical integration of the Cartesian equations of motion of a system with constraints: Molecular dynamics of n-alkanes, *J. Comput. Phys.* 23 (1977) 327-341.
- [19] L. Verlet, Computer Experiments on classical Fluids I. Thermodynamics properties of Lennard-Jones Molecules, *Phy. Rev.* 159 (1967) 98-103.
- [20] A.M. Bonvin, W. F. van Gunsteren, Beta-hairpin stability folding: molecular dynamics studies of the first beta-hairpin of tendamistat, *J. Mol. Biol.* 296 (2000) 255-268.
- [21] S. Jang, S. Shin, Y. Pak, Molecular Dynamics Study of Peptides in Implicit Water: Ab Initio Folding of β -Hairpin, β -Sheet, and $\beta\beta\alpha$ -motif, *J. Am. Chem. Soc.* 124 (2002) 4976-4977.
- [22] A. Porollo, R. Adamczak, J. Meller, POLYVIEW: A flexible Visualization Tool for Structural and Functional Annotations of Proteins, *Bioinformatics* 20 (2004) 2460-2462.