SIMULATION OF FOLDING PATHWAY STUDIES OF TRP-CAGE MINIPROTEIN, AMYLOID A4 PEPTIDE AND α-CONOTOXIN RgIA PEPTIDE

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ABSTRACT

Protein is a sequence of a linear chain of amino acids. Protein folding is a physical process by which the linear chains of amino acid fold into its functional tertiary structures. Misfolding of a protein will lead to the problem such as diseases (cancer and influenza) in protein function. Discovery of protein folding will help the biologist to find the cause of misfolding and also assist the drug designer to find the cure for related diseases. Therefore the objective of this study is to investigate the folding pathways of Trp-cage miniprotein, Amyloid A4 peptide, and α-conotoxin RgIA. The folding process was simulated using molecular dynamics (MD) simulation in both explicit and implicit solvent. Amyloid A4 peptide (350ns) and α-conotoxin (800ns) were simulated in implicit solvent, while the simulation for Trp-cage (150ns) and α-conotoxin (200ns) were performed in explicit solvent method. The simulations produced a huge number of trajectories which were further analysed based on their root mean squared deviation (RMSD) values. The RMSD values showed that these trajectories approaching their simulated native structure (NMR_{MD}). Besides that, a few crucial formations of hydrogen bond, disulfide bond, and salt bridge were involved in stabilizing the folding process. The best structure was identified by clustering all the trajectories based on RMSD, solvent accessible surface area (SASA), van der Waals interaction, electrostatic interactions and total energy of each trajectory. The best structure for Trp-cage miniprotein, Amyloid A4 peptide, α-conotoxin with implicit solvent and, α-conotoxin with explicit solvent were extracted at 79.76 ns, 224.85 ns, 184.20, and 104.20 ns, respectively.

ABSTRAK

Protein merupakan jujukan rantaian asid amino. Lipatan protein adalah satu proses fizikal di mana rantaian lurus asid amino membentuk kepada lipatan struktur tiga dimensi. Kesalahan lipatan protein akan mendorong kepada permasalahan penyakit (kanser dan influenza) dalam fungsi protein. Mengetahui laluan lipatan protein akan membantu ahli biologi untuk mencari punca kesalahan lipatan protein, serta membantu pereka ubat untuk mencari penawar sesuatu penyakit. Oleh itu, objektif kajian ini adalah untuk mengkaji laluan lipatan Trp-cage miniprotein, Amiloid A4 peptide, dan α-conotoxin RgIA. Proses lipatan protein dilakukan dengan menggunakan dua kaedah simulasi iaitu melalui kehadiran air sebagai pelarut dan tanpa kehadiran air sebagai pelarut. Amiloid peptida A4 (350ns) dan α-conotoxin (800ns) adalah protein yang digunakan untuk kaedah simulasi tanpa kehadiran air sebagai pelarut, manakala Trp-cage (150ns) dan α-conotoxin (200ns) telah digunakan dalam kaedah simulasi dengan kehadiran air sebagai pelarut. Proses simulasi menghasilkan banyak trajektori dan ianya telah dianalisa berdasarkan kepada nilai punca min sisihan kuasa dua (RMSD). Nilai RMSD menunjukkan trajektori yang menghampiri struktur sebenar protein. Selain daripada itu, beberapa pembentukan ikatan hidrogen, ikatan disulfid, dan ikatan jambatan garam yang penting telah dikenal pasti membantu menstabilkan proses lipatan protein. Struktur yang terbaik pula telah dikenal pasti dengan mengkelaskan kesemua trajektori berdasarkan RMSD, luas permukaan pelarut boleh capai (SASA), ikatan van der Waals, ikatan elektrostatik, dan jumlah tenaga struktur protein untuk setiap trajektori. Struktur yang terbaik untuk Trp-cage miniprotein, Amiloid A4 peptide, α-conotoxin RgIA tanpa kehadiran air sebagai pelarut dan α-conotoxin RgIA dengan kehadiran air sebagai pelarut telah diekstrak pada 79.76 ns, 224.85 ns, 184.20 ns, dan 104.2 ns.

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LIST OF ABBREVIATIONS

3D	-	Three dimensional structure
AMBER11	-	Assisted model building with energy refinement version 11
CHARMM	-	Chemistry at Harvard molecular mechanics
COM	-	Center of mass
DNA	-	Deoxyribonucleic acid
GB	-	Generalize bond
GROMACS	-	Groningen machine for chemical simulations
MD	-	Molecular dynamics
MMPBSA	-	Molecular modelling poison Boltzmann surface area
MMTSB	-	Multiscale modelling tools for structural biology
NMR	-	Nuclear magnetic resonance
NMR_{MD}	-	Simulated nuclear magnetic resonance structure
PDB	-	Protein data bank
RMSD	-	Root mean square deviation
$RMSD_{c}$	-	RMSD between the best structure
$RMSD_{c\text{-}best}$	-	RMSD between the centroid structure and the best structure
$RMSD_{best\text{-}NMRMD}$	-	RMSD between the best structure and NMR structure.
$RMSD_{c\text{-}NMRMD}$	-	RMSD between the centroid structure and NMR structure
SASA	-	Solvent accessible surface area
VMD	-	Visual molecular dynamics

LIST OF SYMBOLS

%	-	Percentage
Å	-	Angstrom
a_i	-	Acceleration of particle i
E_{kin}	-	Kinetic energy
F_i	-	Force exerted on particle i
K	-	Isothermal compressibility
K_B	-	Boltzmann constant
$l_{i,o}$	-	Bond length
m_i	-	Mass of particle i
N	-	Number of particles
n	-	Number of moles
r	-	Radius
R_i	-	Frictional force
t	-	Time
T	-	Temperature
u	-	Potential energy
α	-	Alpha
β	-	Beta
$\varepsilon_{i,j}$	-	Well depth for Lennad Jone Potential
$\sigma_{i,j}$	-	Collision diameter for Lennard Jones Potential
Φ	-	Dihedral angle in protein structure (phi angle)
Ψ	-	Dihedral angle in protein structure (psi angle)
ω	-	Dihedral angle in protein structure (omega angle)

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Protein is composed of one or more chains of amino acids. Protein carries out important function in every cell. In order for the protein to function correctly, it must fold into its three-dimensional structure. Therefore, understanding the protein folding process is vital because several diseases such as Alzheimer and cancer are directly related to the misfolding of protein. All these diseases have no cure until today and this problem has not been solved for more than 4 decades.

The causes for those diseases such as Alzheimer, Parkinson and Influenza can be found if the folding process of protein is known. This is the major challenge in science today since nobody knows how the protein folds. Theoretically, protein folding is a process in which the sequence of amino acids folds naturally into its three-dimensional structure. The formation of the three-dimensional structure is related to the interaction among amino acid residues. The most important finding in understanding protein folding was carried out by Anfinsen (1972) and his colleagues; they claimed that the structure of the protein is determined by the sequence of amino acids. Findings by Anfinsen and colleagues have inspired researchers to continue investigating the pathways of protein folding. Therefore the evolution in studying protein folding ha increased very rapidly, researchers have come up with various methods and they have proven that protein folding can be simulated using computer (Levitt and Warshel, 1975a). Computational method such as molecular dynamics (MD) simulation is a powerful tool due to its high resolutions and detailed atomic

level representation. Furthermore, the increase in computer speed and improvements in force field along with more efficient computation algorithms have brought realistic computational simulation of the folding process within reach (Pande *et al*., 2003, Scheraga *et al*., 2007).

The aim of this study was to investigate the pathway of the protein folding towards their native or near native state using MD simulation. MD simulation was employed using Amber11 (Case *et al*., 2010). Several studies using this programme shown promising result (Sonavane *et al*., 2008, Best, 2012). There are two types of simulations that can be applied; they are implicit solvent method and explicit solvent method. For this research, both simulations were used. The protein α-Conotoxin (PDB ID: 2JUQ) was simulated using both methods, while Trp-cage (PDB ID: 1L2Y) and Amyloid (PDB ID: 1AML) were simulated using explicit solvent method and implicit solvent method, respectively.

1.2 Problem Statement

Researchers have defined how the amino acid sequence of a protein is coded into DNA. However, the secret on how the protein folds into its three-dimensional structure still remains unsolved. Many theoreticians and biologists have huge interests to investigate the pathway of protein folding. This is proven by the increasing number of new findings on the fundamental, knowledge, and theory of protein folding.

On the experimental front, artificially designed autonomous-folding mini protein has been solved. These findings have helped researchers to address the fundamental issues regarding protein folding. However, protein has marginally stable non-native states that are difficult to observe experimentally. In order to identify this structure, the best method is to use computational simulation. This is because this method has high resolution and provides detailed atomic-level presentations.

1.3 Objective

The objective of this study is to study the folding pathway of Trp-cage miniprotein, Amyloid A4 peptide and α-conotoxin RgIA peptide using molecular dynamics simulation.

1.4 Scopes

There are five scopes for this study. The first scope is to use the Trp-cage miniprotein, Amyloid A4 peptide, and α-conotoxin as subjects for investigating the folding pathway. The second scope is to investigate the hydrogen bond formation, disulfide bond and salt bridge formation from the trajectories. The third scope is to develop clusters from the trajectories based on the RMSD value using clustering analysis. The forth scope is to identify the SASA value for each cluster. The fifth scope is the find the best structure based on RMSD value, radius of gyration, total energy, van der Waals interaction energy, and electrostatic interaction energy.

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