Theoretical studies of the formation mechanism of protein complex by using coarse-grained models

Abstract

Protein is an essential element in every cell of living organisms. Most proteins form a complex to perform its function. The formation of complex system involves protein—protein interaction, which is becoming a great issue in biomolecular studies. In recent times, rapid development on computational study of protein—protein interaction has been greatly advanced by various approaches. In this thesis, coarse-grained models were developed to address several issues, which are unfolding process, crowding effects, and stability of protein complex. Current study will focus on protein azurin, which is known as an extremely stable protein. To observe the stability and dynamics of azurin, structural properties were evaluated. These studies suggested that the Gō-like model is the most accurate model yet lacks transferability. To find more transferable potential model, more physical information are needed for better approach. Hopefully many important insights will be provided by these studies.

Introduction

Protein consists of sequences of amino acids which folds into the unique three-dimensional structure determining its activity. Most folded proteins usually make a protein complex to perform particular function. In recent times, the study of protein dynamics has been rapidly advanced by experimental, theoretical, and computational studies. Computationally, all-atom molecular dynamics simulation has been commonly used to observe the dynamics of protein at the atomic level. However, the all-atom simulations are limited to small systems and nanosecond time scales.

In the last decade, coarse-grained models have gained much attention since they could overcome the limitations of all-atom model. Basically, coarse-grained model in molecular dynamics is a lower resolution model where some of atomic details are eliminated so that coarse-grained models allow us to simulate larger systems and slow processes where micro- to millisecond time scales are required. For instance, native center model, pioneered by Gō [1], has retained a great success on studying the folding mechanism by minimizing topological frustration.

In this thesis, we study the formation of protein complex by using coarse-grained models. First, the off-lattice Gō model is implemented to investigate the unfolding dynamics of single protein. Then, the coarse-grained model is improved by considering the protein-protein interaction to be applied for a larger system. This research will focus on *Pseudomonas aeruginosa* azurin. Azurin from *Pseudomonas aeruginosa* is known to exhibit a large stability [2] and functions in the electron transfer. Later, azurin also has been considered as a proper candidate for treatment of cancer through nanotechnology. Hopefully this thesis will provide valuable insights into the study of the formation of protein complex, in particular azurin complex.

Material and Methods

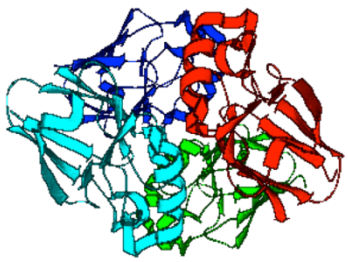

Fig 1. Three-dimensional structure of *Pseudomonas aeruginosa* azurin (4AZU)

The native conformation of azurin was taken from the X-ray crystal structure of wild type azurin with PDB entry 4AZU [3]. This azurin complex consists of a tetramer of identical azurin molecules (see Fig. 1), where each azurin is composed of eight β-strands and one α-helix. Each amino acid is treated as a single bead represented by the C_α position.

The potential energy for the entire system is distinguished into intramolecular and intermolecular interactions. The individual chain will be treated as rigid as possible. Therefore, Gō-like model (see Eq. 1 and 2) [4] is applied to represent the intramolecular interaction. Meanwhile, the implementation of two intermolecular potentials will be compared, which are the non-bonded Gō-like model (Eq. 2) and the widely used Lennard-Jones potential (Eq. 3) [5].

$$E_{bonded|go} = K_{bond}\left(r - r_0\right)^2 + K_\theta\left(\cos\theta - \cos\theta_0\right)^2 + K_\phi\left[1 - \cos\left(\phi - \phi_0\right)\right] + K_\phi \Big/ 2\left[1 - \cos\left(3 \times \left(\phi - \phi_0\right)\right)\right] \quad (1)$$

$$E_{non-bonded|go} = \varepsilon_{nat}\left[5\left(\frac{r_0^{ij}}{r_{ij}}\right)^{12} - 6\left(\frac{r_0^{ij}}{r_{ij}}\right)^{10}\right] + \varepsilon_{non-nat}\left(\frac{C}{r_{ij}}\right)^{12} \quad (2)$$

$$E_{LJ} = 4\varepsilon_{LJ}\left[\left(\frac{\sigma}{r_{ij}}\right)^{12} - \left(\frac{\sigma}{r_{ij}}\right)^{6}\right] \quad (3)$$

The parameters are set to be: K_{bond} = 100 kcal/mol/Å^2, K_θ = 20 kcal/mol, K_ϕ = 1 kcal/mol, $\varepsilon_{nat} = \varepsilon_{non-nat}$ = 1 kcal/mol, ε_{LJ} = 0.13 kcal/mol, C = 4.0 Å and σ = 6.5 Å. The beads are coupled to Langevin thermostat to mimic the non-conservative forces from the surrounding solvent and control a constant temperature at 300 K. The friction coefficient is set to be ζ= 0.5 (τ^{-1}) where τ, a time unit, is 3 ps.

For the analyses, in order to study the effects of protein—protein interactions to the stability of azurin complex, some static and dynamical properties were calculated from the simulated trajectories, such as root mean square displacement, surface area, and intermolecular contacts.

Results

First, a coarse-grained simulation of a single azurin was performed to observe the unfolding process of wild-type azurin and mutated azurin (H117G). Under thermodynamic conditions most of the folding process is known as a two state reaction. In such a case, the free energy profile has double minimum corresponding to the ensembles of native state and denatured state.

To obtain the free energy profile, the thermodynamic configurations were observed as a function of nativeness, Q, along simulation time. At the folding temperature (T_f), both of the wild-type and H117G azurin simulations indicate the two state reactions as shown in Fig 2. This result is in agreement with the experimental measurements where both of azurins unfold in two-state without intermediates and the mutation of His117 to Gly affects the stability of azurin [6].

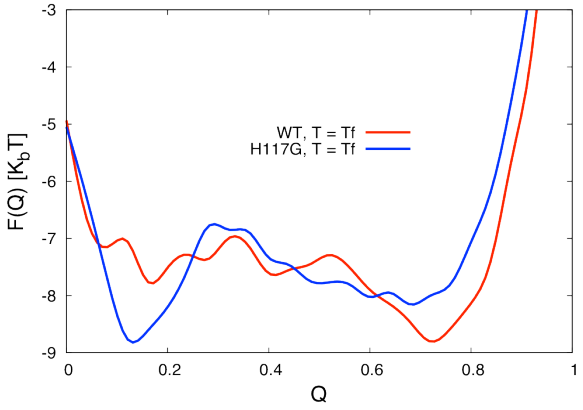

Fig 2. Free energy profiles of wild-type (red) and mutated azurin (blue)

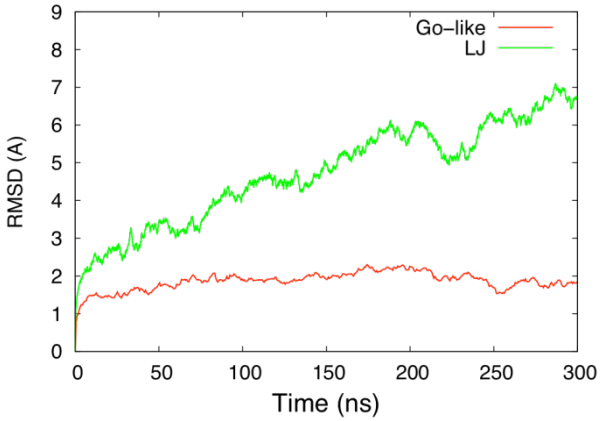

Fig 3. RMSD profiles of tetramer azurin

In the next step, coarse-grained models for azurin complexes were developed. First, the non-bonded interaction of the Gō-like model (Eq. 2) was adopted into the intermolecular interaction in azurin complex. This potential is suitable to maintain the stability of protein complexes and is able to reproduce the tetramer azurin since this model minimizes the topological frustration. Nevertheless, this potential lacks transferability because it can be used only for a particular system.

To overcome this limitation, more general potential model was explored by adopting the widely used Lennard-Jones potential (Eq. 3). However, it is very difficult to determine the parameters. Even though the intermolecular interaction parameter satisfies the limitation that intermolecular interaction should be weaker than intramolecular interaction, this approach has been found not so accurate yet to reproduce the azurin complex. Fig. 3 clearly shows that on the second model, the conformation of tetramer azurin changes a lot. The increase of intermolecular contact indicates that there is a tendency for the tetramer to aggregate (data is not shown).

Overall, it is found that Gō-like model is a more realistic model to represent the interaction of protein complex. It shows that even for the intermolecular interaction, the native structure plays a significant role. This study suggested that the term σ can not be oversimplied. The intermolecular interaction, especially in the binding area, need to be treated more carefully by considering more physical informations from the crystal structure.

Conclusion

In this thesis, the formation mechanism of azurin complex was observed by using coarse-grained models. It begins with a coarse-grained simulation of a single azurin via implementation of Gō-like model to observe the unfolding process of wild-type azurin and H117G azurin. The mutation of His117 to Gly was found to affect the stability of the denatured state and the mutated azurin unfolds faster than the wild-type as expected.

For the main purpose of this thesis, two intermolecular potentials were developed. Nevertheless, developing accurate and transferable coarse-grained potential for protein complex remains a challenge. Intermolecular interaction in protein complex often can not be derived into a simple model. It has to be carefully determined which phenomena can be simplified and which should be described with more complicated models.

In the future, knowledge-based approaches can be considered. Physical informations from known PDB structure should be employed to develop a set of transferable and more appropriate interactions for azurin complex. When this problem is addressed, it would significantly improve the scope of coarse-grained protein model to be able not only to reproduce known structure but also to predict the dynamics of unknown structure.

References:

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学位論文審査報告書（甲）

１．学位論文題目（外国語の場合は和訳を付けること。）

Theoretical Studies of the Formation Mechanism of Protein Complex by Using Coarse-grained Models

（粗視化モデルを用いたタンパク質複合体形成機構に関する理論的研究）

２．論文提出者　(1) 所　　属　数物科学　専攻

(2) 氏（ふり）名（がな）　Micke（みけ） Rusmerryani（るすめりあに）

３．審査結果の要旨（600～650字）

当該学位論文に関して、各審査員が個別に検討し面接調査を行った後、論文内容を詳細に検討した。その後平成２６年７月３１日に行われた口頭発表の後に審査委員会を開き、協議の結果以下のように判定した。

本論文はタンパク質の粗視化モデルを用いて具体的に銅タンパク質アズリンの複合体形成を引き起こすタンパク質間有効相互作用のモデル化に関する理論的研究を行っている。アズリンは緑膿菌に存在し、離れた位置にあるタンパク質に電子を運搬する電子伝達機能を有するタンパク質である。電子運搬に於けるタンパク質間の会合解離に関わるタンパク質間有効相互作用の解析は理学や医薬学分野においても意義深い。Go モデルを用いてアズリンのフォールディングに関する解析を行い、実験結果を再現するタンパク質内の残基対に対する安定化エネルギーが約 1kcal/mol となることを示した。また、X 線構造解析で得られたアズリン複合体構造からタンパク質間有効相互作用のモデル化を行った点は新しい。複合体形成を再現するためには、タンパク質間で相互作用する残基対に対する安定化エネルギーが約 0.2kcal/mol となり、タンパク質内の残基対のものより小さくなることを示した。さらにタンパク質間の残基対間平衡距離がタンパク質内の残基対のものより長いことも示した。この一連の研究結果は先行研究とも矛盾せず、構築したモデルの有効性を示すと伴に、今後の理論や実験研究にも多くの寄与をもたらすものである。

以上により、この論文は博士（理学）に値するものと判断した。

４．審査結果　(1) 判　　定（いずれかに○印）（合　格）・　不合格

(2) 授与学位　博　士（　理学　）