様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Computational investigation of the role of disordered regions in enzymatic reactions

研究課題名（英文）Computational investigation of the role of disordered regions in enzymatic reactions

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　2,000,000円

研究成果の概要（和文）：酵素反応における本質的無秩序領域（IDR）の役割を、マルチスケール計算機手法を用いて明らかにした。古典的、MDの結果、IDRがChrosimate Mutaseの活性部位に大きな揺らぎをもたらし、反応性複合体を形成するために必要な基質の再編成を可能にすることが示されました。また、QM計算により、IDRが反応障壁を下げる役割を担っていることを確認しました。この結果は、酵素反応における構造的無秩序の未知の役割に光を当てるものである。さらに、ドメイン運動を促進する新しい方法を提案し、SARS-CoV-2の構造転移の研究に応用した。SARS-CoV-2の構造変化の研究に応用した。

研究成果の学術的意義や社会的意義
Enzymes are the best catalysts that accelerate chemical reactions. This project clarify the role of disorder and propose a computational approach to study disorder-function paradigm in Enzymes. A new method was also proposed and applied to study structure changes in Spike protein of SARS-CoV-2

研究成果の概要（英文）：The role of intrinsically disordered region (IDR) in enzymatic reactions was elucidated, using multiscale computational approach. This includes classical and enhanced sampling molecular dynamics (MD) simulations and Quantum mechanical (QM) calculations. MD results show that IDR induces large fluctuation in an engineered Chrosimate Mutase active site, allowing for a necessary substrate reorganization to form reactive complex. QM calculations confirmed the role of IDR in lowering the reaction barrier. The results shed the light on an unrecognized role of structural disorder in enzymatic reactions. Furthermore, new method to enhance domain motion was proposed and applied to study conformational transition in SARS-CoV-2.

研究分野：Computational Biochemistry

キーワード：IDR　Protein dynamics　enhanced sampling

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

The modern discovery of Intrinsically disordered proteins (IDPs) has led to a new concept of disorder-function paradigm in protein science. IDPs constitute a large fraction of the proteome (44% in human) and play central roles in numerous biological processes. Enzymes have long been considered as an exceptional class of proteins that might not include intrinsically disordered regions (IDRs), as structural disorder is generally expected to reduce catalytic efficiency. Notably, recent experimental studies have established the occurrence of IDR in more than one hundred enzymes, so far.

The functional significance and the roles of IDR formation in enzymes are generally poorly understood. Few studies have indicated the importance of IDR to maintain high catalytic rate. For instance, increasing the rigidity or flexibility of IDR in *Sporosacina pasteurii* UreG enzyme reduces its catalytic efficiency. Furthermore, the introduction of disordered region in an engineered monomer *Methanococcus jannaschii* chorismate mutase (mMjCM) retains high catalytic rate, comparable to the structurally ordered MjCM dimer.

The current emerging field of IDR enzymes provide a new aspect of enzymatic function that uses an ensemble of conformers of disordered regions to optimize/regulate their catalytic rates, via an unknown mechanism. Consequently, new computational techniques to explore IDR dynamics in enzymatic reactions is needed. In this project, I aimed to establish a computational approach to unravel the effect IDR dynamics on enzymatic reactions, using mMjCM as an example. Furthermore, the goal of the original studies was expanded to suggest an effective enhanced sampling approach to widely sample even larger conformational changes in proteins, using ribose binding protein (RBP) as a benchmark. The same method was successfully applied to study active to inactive conformational transition in Spike (S) protein of SARS-CoV-2.

２．研究の目的

First, to unravel the catalytic role of IDR in mMjCM using multiscale computational approaches. Second, to link the effect of IDR on enzyme dynamics and the enzyme catalytic reaction. Third, to propose a new computational method that allow for wide sampling in IDR enzymes and protein in general.

３．研究の方法

To study the structural effect of IDR on mMjCM active site and substrate binding/reaction, the results of atomistic molecular dynamics (MD) simulations were analyzed. Both classical and enhanced sampling (the generalized replica exchange with solute tempering (gREST) MD approaches were used. Quantum mechanical calculations were used to investigate pre-reactive and reactive complex of chorismate. A new enhanced sampling approach based on the selection of surface charged residues only in gREST (gREST_SSCR) was proposed and applied to enhance domain motion achieving wide sampling in both RBP and S-protein.

４．研究成果

(1) The effect of IDR on mMjCM catalysis:

To elucidate the effect of IDR on mMjCM active site, two classical molecular dynamics simulations (cMD) were performed. First, the solvated mMjCM/chorismate complex were simulated for 1 μs, without applying any restraints. Secondly, a 500 ns simulation was performed upon applying a weak force of 1.0 kcal mol^{-1} $Å^{-2}$ on the Cα atoms of the protein, using the root mean square deviation (RMSD) restraint function in GENESIS software. Such restraint was employed to reduce IDR fluctuation, preserving the protein conformation closer to the initial NMR structure (PDB:2gtv). Additionally, a third simulation was performed using gREST enhanced sampling approach using 8 replicas that cover temperature range from 300 to 558 K. All atoms of IDR residues were selected as solute region and each replica was simulated for 500 ns, with a total of 4 μs.

Comparison of the three simulations show that the restraint MD(cMD(rest)) kept the protein RMSD around 2Å. While both cMD and gREST results shows larger values with an average of 3.7Å. Similarly, the radius of gyration was also increased, suggesting IDR induces motion in the whole protein.

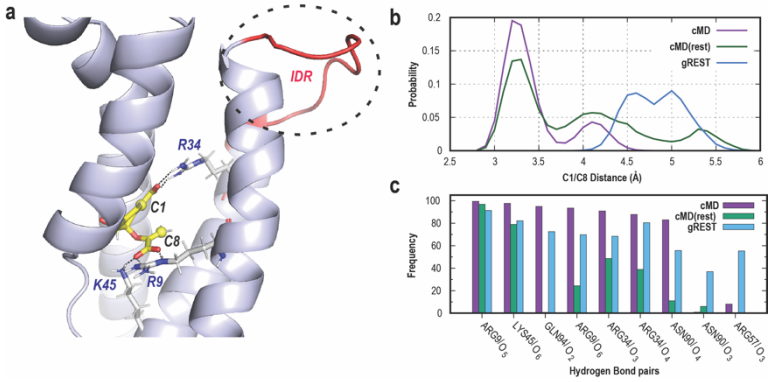

Figure 1. a) Structural representation of mMjCM complex, showing ligand interactions. Probability distribution of C1/C8 atoms distance (b), and the ligand hydrogen bonding interactions (c), in all 3 simulations.

To understand how IDR might affect the distant catalytic site, I compared ligand organization and interactions (Figure 1). Figure 1b show that cMD(rest) has large C1/C8 distance that would hinder the catalytic reaction. While cMD results show significant distribution around 3 Å, forming reactive complex. Notably, gREST simulation at 300 K were able to sample all conformations. Figure 1c, shows that the reduction in C1/C8 distance occur due to the formation of multiple hydrogen bonding interactions with the ligand. These interactions are maximized through the IDR induced motion in the active site. Note that QM calculations also show the higher stability of larger C1/C8 distance (3.8 Å) in the absence of the active site, which emphasize the role of active site interactions in forming the reactive complex. In summary, the results show the role of IDR in inducing a breathing like motion that maximize ligand interactions, leading to a reduction in reaction barrier. It also shows the superiority of gREST method and its suitability for studying IDR enzymes.

(2) Enhancing domain motion:

Considering the small size of mMjCM, the application of gREST using all atoms was feasible. However, as the protein size increases the solute region might also increase, limiting gREST application. As a result, I proposed gRSET with selected surface charged residue (gREST_SSCR), wherein a small number of residues/parameters can be selected regardless of the protein size. This approach was first tested in RBP showing the conformational transition from close to open, within only 250 ns (1). The obtained transition pathway and intermediates were further validated using cMD simulations and free energy calculations (2).

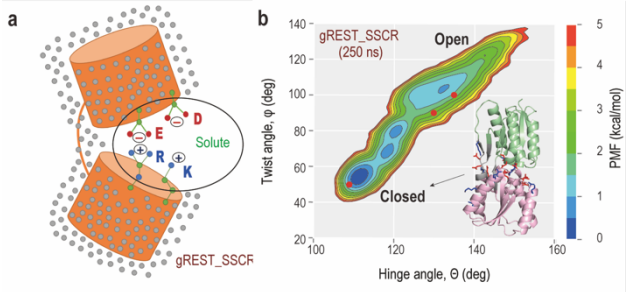

Figure 2. a) Schematic representation of solute selection approach in gREST_SSCR. b) Free energy map along hinge and twist angles showing transition pathway obtained for RBP.

3) Conformational transition in Spike protein of SARS-CoV-2:

In response to COVID-19, I applied gREST_SSCR to study Spike motion starting from Down and Up conformations (3,4). Wherein we sampled Down to 1Up and 1Up to 2Up transitions. Our results agree with Cryo-EM structures and smFRET experiment. Furthermore, using pocket search algorithm and virtual screening, I identified unprecedented cryptic pocket at RBD interface in the intermediate states, that might be targeted to shift spike dynamics toward Down conformation, reducing cell entry.

(1) Dokainish, H. M.; Sugita, Y., *Int J Mol Sci* **2020,** *22* (1), 270.

(2) Ren W*., Dokainish H. M*., Shinobu A., Oshima H., Sugita Y. *J. Phys. Chem. B,* **2021**, 125(11), 2898-2909. (**First co-author*)

(3) Dokainish, H. M, Suyong Re, Takaharu Mori, Chigusa Kobayashi, Jaewoon Jung, Yuji Sugita. *eLife,* **2022**. 11:e75720.

(4) Dokainish, H. M, Suyong Re, Takaharu Mori, Chigusa Kobayashi, Jaewoon Jung, Yuji Sugita. *Biophys. J.* **2022**, 121(3), 457a.

５．主な発表論文等

〔雑誌論文〕　計5件（うち査読付論文　3件／うち国際共著　5件／うちオープンアクセス　4件）

１．著者名 Dokainish Hisham M.、Sugita Yuji	４．巻 22
２．論文標題 Exploring Large Domain Motions in Proteins Using Atomistic Molecular Dynamics with Enhanced Conformational Sampling	５．発行年 2020年
３．雑誌名 International Journal of Molecular Sciences	６．最初と最後の頁 270～270
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/ijms22010270	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Ren Weitong、Dokainish Hisham M.、Shinobu Ai、Oshima Hiraku、Sugita Yuji	４．巻 125
２．論文標題 Unraveling the Coupling between Conformational Changes and Ligand Binding in Ribose Binding Protein Using Multiscale Molecular Dynamics and Free-Energy Calculations	５．発行年 2021年
３．雑誌名 The Journal of Physical Chemistry B	６．最初と最後の頁 2898～2909
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acs.jpcb.0c11600	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Dokainish Hisham M、Re Suyong、Mori Takaharu、Kobayashi Chigusa、Jung Jaewoon、Sugita Yuji	４．巻 11
２．論文標題 The inherent flexibility of receptor binding domains in SARS-CoV-2 spike protein	５．発行年 2022年
３．雑誌名 eLife	６．最初と最後の頁 e75720
掲載論文のDOI（デジタルオブジェクト識別子） 10.7554/eLife.75720	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Dokainish Hisham M.、Re Suyong、Mori Takaharu、Kobayashi Chigusa、Jung Jaewoon、Sugita Yuji	４．巻 121
２．論文標題 Unraveling SARS-CoV-2 spike protein activation pathway reveals unprecedented cryptic pockets	５．発行年 2022年
３．雑誌名 Biophysical Journal	６．最初と最後の頁 457a～457a
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.bpj.2021.11.491	査読の有無 無
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Dokainish Hisham M.、Sugita Yuji	４．巻 -
２．論文標題 Structural Ramifications of Spike Protein D614G Mutation in SARS-CoV-2	５．発行年 2022年
３．雑誌名 BioRxiv	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1101/2022.01.24.477651	査読の有無 無
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 Hisham M. Dokainish
２．発表標題 Unraveling SARS-CoV-2 spike protein activation pathway reveals unprecedented cryptic pockets
３．学会等名 The Biophysical Society Annual Meeting（国際学会）
４．発表年 2022年

１．発表者名 Hisham M. Dokainish
２．発表標題 Extensive Sampling of Spike protein down, one-up, one-open, and two-up-like Conformations and Transitions in SARS-Cov-2.
３．学会等名 The Biophysical Society of Japan Meeting
４．発表年 2021年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関