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学位（専攻分野）	博士　(理学)
学 位 記 番 号	総研大甲第 1405 号
学位授与の日付	平成２３年３月２４日
学位授与の要件	物理科学研究科　機能分子科学専攻 学位規則第６条第１項該当
学 位 論 文 題 目	Molecular Recognition Process of Biological Molecules Studied with a Statistical Mechanics Theory
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論文内容の要旨

The Molecular Recognition (MR) in living systems is a crucial elementary process for biomolecules to perform their functions as, for example, enzymes or ion channels. The MR process can be defined as a molecular process in which one or few guest molecules are bound in high probability at a particular site, a cleft or a cavity, of a host molecule in a particular orientation. The process is governed essentially by the two physicochemical properties: (1) difference in the thermodynamic stability (or free energy) between the bound and unbound states of host and guest molecules, and (2) structural fluctuation of molecules. In the dissertation, the author proposes a new theory to describe the molecular recognition process based on the statistical mechanics of molecular liquids.

The new theory of MR referred to as uu-3D-RISM is formulated in Chapter II, after a brief sketch of the three-dimensional reference interaction site model (3D-RISM) theory, a statistical mechanics theory of molecular liquids, on which the new theory of MR is based. The 3D-RISM theory itself has been applied successfully to a variety of MR problems in the last five years in Hirata's group. He himself applied the theory to binding of small ligands, such as CO and O_2, in myoglobin. (The study is presented in the following chapter.) However, the 3D-RISM equation had some technical problem when it is applied to a larger ligand typically used as a drug. The new theory overcomes the problem, and can be applied to larger organic molecules.

Both the 3D-RISM and uu-3D-RISM equations are derived from the molecular Ornstein- Zernike (MOZ) equation, the most fundamental equation to describe the density pair correlation of liquids, for a solute-solvent system in the infinite dilution by taking a statistical average over the orientation of solvent molecules. By solving combined the 3D-RISM with RISM equations, the latter providing the solvent structure in terms of the site-site density pair correlation functions, one can get the "solvation structure" or the solvent distributions around a solute. The high peak of the solvent distributions indicates that the solvent affinity of target protein or receptor at that point is high. Therefore, the MR can be realized by the theories in terms of the distribution of solvent or ligand just like in the X-ray crystallography. The method produces naturally all the solvation thermodynamics as well, including energy, entropy, free energy, and their derivatives such as the partial molar volume and compressibility.

In the all previous studies of MR due to the 3D-RISM theory, the receptor protein and ligand molecules were regarded as solute and solvent, respectively. In those cases, the MR process is analyzed in terms of solvent distribution around solute molecule, which is called as sol'u'te- sol'v'ent distribution function (uv-DF). However, it is still difficult technically to treating a large ligand molecule as solvent in terms of numerical convergence. Therefore, He proposes a new approach to tackle the MR of

large ligand molecules by protein based on the 3D-RISM and RISM theory. The strategy of the method is to regard a ligand molecule as solute which is immersed in solvent in the infinite dilution limit in addition to a receptor protein. The distribution of ligand molecule around a receptor protein is described by the sol'u'te-sol'u'te distribution function (uu-DF) instead of sol'u'te-sol'v'ent DF (uv-DF). In this sense, the new method is named uu-3D-RISM. Under the treatment of this method, interactions between ligand molecules are completely omitted, because the density of ligand molecule vanishes at the limit. Therefore, it is not necessary to solve the ligand-ligand RISM equation, most unstable equation, anymore. This assumption stabilizes the numerical solutions of a set of the 3D-RISM and RISM equations dramatically.

In Chapter III, the molecular recognition of small ligands to myoglobin is studied by using the original 3D-RISM theory. The Chapter consists of two sections. The first section treats the binding affinity of small ligands including O_2, Xe, NO, CO, H_2S, and H_2O to myoglobin. Those ligands are known to show some physiological activities in living bodies, such as anesthetics, poisons or signal transducer. Although it is not entirely clear how the affinity of these ligands to cavities inside the myoglobin is related to the physiological activities, it is worthwhile to find out the factors to determine the selectivity of the ligands to the cavities to provide basic molecular information to the physiology. The affinity is evaluated in terms of the coordination number of the ligand molecules in cavities in the protein, or the "Xe site," which can be obtained from the radial distribution of ligands inside the cavities. It was found that NO, CO, and H_2S show greater affinity to the Xe-sits than O_2 does, while the affinity of Xe is lower than that of O_2.

The second section concerns the CO escaping pathway of myoglobin. The CO dissociating process occurs from heme to solvent through some specific cavity. The CO escaping pathway from myoglobin was discussed in terms of partial molar volume change along the pathway. The results showed excellent agreement with those from the transient grating experiments carried out by Terazima and his coworker.

In Chapter IV, the new methodology, or uu-3D-RISM, described in the chapter II, is applied to two types of proteins, the structure of which can be available in the Brookhaven Protein Data Bank (PDB).

One is the odorant binding protein LUSH, which can form a complex with a series of short-chain n-alcohols, from Drosophila melanogaster. It clears a set of molecular interactions between the protein and the alcohol at a specific alcohol-binding site. In order to prove the robustness of the new method, both the original 3D-RISM and the new method is applied to this system.

The other example is Phospholipase A2 (PLA2) enzyme which can form the complex with 2-acetoxybenzoic acid, a compound well known as "aspirin." Aspirin induces its anti- inflammatory effects through its specific binding to PLA2. PLA2 is potentially an

important target for structure-based rational drug design. Aspirin is embedded in the hydrophobic environment and several important attractive interactions are formed with protein. Our calculation clearly shows that aspirin occupies a favorable place in the specific binding site of PLA2.

The results for the both proteins demonstrate that the new theory is a powerful tool to describe the molecular recognition process of biomolecules in living system.

博士論文の審査結果の要旨

タンパク質をはじめとする生体分子の機能は分子認識を通じて発現される。本論文は統計力学に基づいて生体分子の分子認識の仕組みを理解するための理論的アプローチを記述するものである。

第1章では、分子認識を理解するうえで生体分子の水和と揺らぎを考慮することの重要性が提起されており、連続誘電体モデルに基づく方法や分子シミュレーション法など、これまで広く用いられているアプローチ法の問題点が提示されている。こうした観点に基づき、本論文の学術的位置づけが述べられている。

第2章では、水和を考慮した分子認識を統計力学的原理に基づいて取り扱う3D-RISM/RISM理論について詳細な説明がなされている。従来の3D-RISM理論は薬物のような大きなリガンド分子への適用が困難である。本論文ではこの問題を克服するために、レセプタータンパク質とリガンドの双方を溶質と見做して両者の分布関数を取り扱う新たな理論（uu-3D-RISM）を提案し、定式化している。

第3章では、3D-RISM法を用いてミオグロビンにおける種々な低分子リガンドの結合部位を予測し、COの分子内の離脱経路を解析することに成功している。この結果は、すでに報告されている実験結果とも見事な一致を示している。さらに、得られた結果について分子科学的な観点から明快に考察している。

第4章では、本論文で新たに提案されたuu-3D-RISM法を、ショウジョウバエの匂い物質結合タンパク質とエタノール分子との結合およびホスホリパーゼA2と薬物（アスピリン）との結合の解析に成功裡に応用している。これにより、分子認識におけるリガンド分子内の電荷分布の重要性など、薬物設計に示唆を与える知見を得ている。

第5章では、全体の総括と今後の展望が述べられている。特に、生体分子のコンフォーメーションの揺らぎを取り扱うことなど、今後に残された課題についても言及されている。

以上のように、本論文はこれまで理論的に取り扱うことが困難であった生体分子の分子認識に果敢に挑戦して、新しいアプローチ法を確立することに成功している。本論文の成果は分子科学に新機軸をもたらし、さらに薬物設計にも新たな指針を与えるものとして高く評価することができる。また、本研究の成果の一部は、出願者を筆頭著者とする欧文論文として *Journal of the American Chemical Society* など2報の学術雑誌に公表されている。以上の点に鑑みて、審査委員会は本論文が博士（理学）の学位授与に値すると全員一致で結論付けた。