Heterotetramic sarcosine oxidase [SO, EC 1.5.3.1] catalyses the oxidative demethylation of sarcosine to generate glycine, hydrogen peroxide, and formaldehyde or 5,10-methylene-tetrahydrofolate, depending on the availability of tetrahydrofolate. SO was first purified from *Corynebacterium* sp. U-96 [SO-U96], and then from various sources. SO-U96 comprises four non-identical subunits (α , 110 kDa; β , 44 kDa; γ , 21 kDa; δ , 10 kDa) and one FAD, one FMN, and one NAD⁺. The 3D structure of the SO-U96-complex with dimethylglycine [DMG] has been determined from X-ray crystallography. The structural and biochemical analyses gave new insight into the cavity, conformational change, and channeling of oxygen, sarcosine, and products through the cavity. The authors performed the free energy perturbation molecular dynamics simulations in which the bound DMG was mutated by the coupling parameter approach into sarcosine, and analysed the simulated enzyme-substrate complex from the structural and energetic viewpoints. The mobility of substrate was also analyzed to examine the possibility of realistic simulations of channeling of substrate and products. The GROMACS program, the GROMOS 53a6 energy parameters, and the SPC water parameters, and the particle mesh Ewald method were used. The numbers of the atoms were from 130 000 to 160 000, depending on the number of the water molecules included in the simulations.

1PT176 アデニル酸キナーゼ反応機構に関する ONIOM 法による研究

Study on the reaction mechanism of adenylate kinase with ONIOM method

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Adenylate kinase catalyzes the reaction: ATP + AMP + Mg²⁺ → ADP + ADP + Mg²⁺. In order to elucidate the nature of the reaction, we have been studying the theoretical model using MM/QM. We constructed the complex structure of enzyme and substrates, ATP and AMP with Mg ion, based on the PDB data of complex with inhibitor. After the full optimization of the whole molecule in vacuo with MM, we put TIP3P water molecules around catalytic center Mg ion with spherical boundary condition, and harmonic constraint potentials on the surrounding atoms in the boundary region. Some model structures were sampled from 900 K MD simulation followed by structural energy minimizations. In order to obtain the reasonable initial model of the transition state, we performed the molecular mutation calculation from the reactant to the product. Further truncated models were used for the calculations with ONIOM method, the reactant, product, transition structures were optimized. In these calculations, small barrier (several kcal/mol) shown in the low level model (19/834 atoms in QM/MM, HF/6-31G(d):AMBER) vanishes with higher level, and the present highest model (89/764 atoms in QM/MM, B3LYP/6-31+G(d):AMBER, embed model) gives the reaction barrier of 19 kcal/mol, and 10 kcal/mol exothermicity. For further discussion, however, more qualitative consideration as well as free energies on the conformational effects of the surrounding atoms is necessary.

1PT177 酵素の多機能性に関する解析

Analysis of enzyme promiscuity

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For a long time, enzymes are believed to catalyze specific reactions with specific substrates. Specificity is essential for enzymes to work in the crowded environment of a cell. However, recent experimental studies have shown that many enzymes can catalyze more than one reaction or have broad substrates (i. e., enzyme promiscuity). It is becoming recognized that enzyme promiscuity is related to functional evolution, drug metabolism and protein engineering [1]. Although molecular mechanisms of promiscuity of each enzyme have been investigated, there have only been a few attempts to define the general properties that characterize promiscuous enzymes, and no comprehensive analysis using reaction constants have been made [2]. Moreover, a good measure for describing promiscuity has not been established.

In order to elucidate how widespread the substrates and reactions are that an enzyme can bind and catalyze, we performed a systematic analysis of the enzyme data in the BRENDA database [3]. The enzymes 1) for which UniProt ids were obtained from BRENDA or through PubMed ids and NCBI Entrez links, and 2) which have Michaelis constants or turnover numbers for at least ten different substrates were selected for the analysis. At the fourth level of Enzyme Commission Number and within an orthologous enzyme group, the relationships among sequence identities, reaction constants and substrate similarities are discussed.

1. Nat Biotechnol 2009;27:157. 2. Biochemistry 2008;47:157. Bioinformatics 2010;26:2012. 3. Nucleic Acids Res 2009;37:D588.

1PT178 Folding of topologically complex proteins by an atomic interaction based coarse grained model

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While fast folding of small proteins has been relatively well characterized by experiments and theories, much less is known for slow folding of larger proteins. Recent progress of experimental works on the folding of large proteins revealed a number of new features arising from the topological complexity, which poses new challenges to the current protein folding theories. Reasonably describing the folding of such topologically complex proteins needs more sophisticated theoretical models. In this presentation, we introduce a new model, i. e., AICG model, developed within the framework of the energy landscape theory. The features of this new model include: i) contact interactions are delicately weighted according to the atomic interactions via a multiscale protocol; ii) a realistically flexible energy function is used for the local interactions. We applied this model to a number of topologically complex proteins, including multi-domain proteins and knotted proteins, and it reproduced all the major features of folding revealed experimentally. Our results suggest that the structure-based model can capture the essential features of the folding of proteins with complex topologies after delicately weighting the contact interactions, and including realistic local flexibilities.

1PT179 多様な分子コンホメーションを取り得る溶質の小角 X 線散乱プロフィールの近似計算法

A new approximation method for estimating SAXS profiles of solutes with multiple molecular conformations

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Small-angle X-ray scattering (SAXS) has long been used as an important means for determining global-structure parameters of biopolymers in solution. It can be applied not only to natively globular proteins with a practically single global structure but also to natively unfolded proteins with many available conformations such as intrinsically disordered proteins (IDP). In the latter case, however, we need to evaluate a large number of SAXS profiles for averaging them, because the IDP consists of an ensemble with diverse conformations. The program CRYSOLE (Svergun, D. et al., *J Appl. Cryst.*, 1995) has been commonly used to calculate SAXS profiles of IDPs. We found that the calculated profiles contain a non-negligible amount of error caused by the approximation taken in estimating the contribution of protein hydration-layer. Though it is possible in principle to use the method combined with MD simulation (Seki, Y. et al., *Biophys. Chem.*, 2002) for removing the error, the computational cost would go impractically high for IDPs. We present here a new approximation method for estimating SAXS profiles applicable to solutes with multiple molecular conformations. Though the accuracy of this method is lower than that of the MD-combined method in the large-K region, it gives sufficient accuracy in small-K region with low computational cost. In the meeting, we will report on the detail of approximation and its applicable range of scattering angle.

1PT180 極低温電子顕微鏡による 26S プロテアソームの単粒子解析

Single particle analysis of 26S proteasome by cryo-electron microscopy

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The eukaryotic 26S proteasome functions to degrade a large fraction of intracellular proteins. The 26S proteasome is composed of at least 33 different subunits in 20S core particle (CP) and one or two 19S regulatory particles (RPs). Proteins destined for degradation are marked by poly-ubiquitin, a degradation signal that is recognized by the RPs. The proteolytic sites are sequestered inside the CP and are accessible only through a narrow channel. Thus the RP deconjugates ubiquitin chains, unfolds substrate proteins, and translocates them into the catalytic CP. The CP was amenable to structure determination by X-ray crystallography but the 26S holocomplex with 1 or 2 RPs has so far resisted all crystallization attempts. Here we optimized the single particle analysis methods by cryo-electron crystallography for the structural analysis of the 26S proteasome.