

Biofunctional Chemistry Research Section

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1. Introduction

A transition to renewable energy technologies requires new chemistry to learn from nature. Nature has developed fantastic solutions to convert the solar energy to the chemical energy and to utilize them in the exceptionally efficient manners for almost 3 billion years. It is our challenge to understand the efficient bioenergetic processes of nature and to construct bio-inspired energy utilization systems. The research interests in our group focus on the design of biomacromolecules and their assemblies for molecular recognition, catalysis and signal transduction in water, the solvent of life. We take synthetic, organic chemical, biochemical and biophysical approaches to understand the biological molecular recognition and chemical reactions. Proteins and protein/nucleic acids assemblies are explored to realize biomimetic function of biological systems, such as visualization of cellular signals by fluorescent biosensors, directed self-assembly of peptides and proteins to build up nanobiomaterials, tailoring artificial receptors and enzymes based on the complex of RNA and a peptide or a protein, and reconstitution of the functional assemblies of receptors and enzymes on the nanoarchitectures. Followings are main research achievements in fiscal year 2020.

2. Enhanced enzymatic activity exerted by a packed assembly of a single type of enzyme

Enzymes are often spatially organized within the cell, either in close proximity on the cell membrane or confined inside a micro-compartment. Such environments are believed to play key roles in enabling the extraordinary efficiency and specificity of sequential metabolic enzymatic reactions. A typical example is the hetero oligomeric assembly of enzymes. Compartmentalization also regulates the spatial organization of enzymes. In spite of being crucial for cellular functions, enzymatic reactions in such highly packed states have not been fully addressed.

In this work, we apply a protein adaptor to assemble a single type of monomeric enzyme, carbonic anhydrase, on a DNA scaffold in a packed state with less

than a few nanometers inter-enzyme distance or dispersed state and show that the esterase reaction proceeds faster in the packed than in the dispersed state (Figure 1).

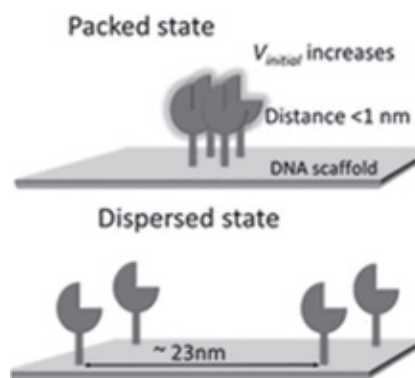


Fig. 1 Schematic representations of four ZS-CAs in the packed (upper image) and dispersed (lower image) states.

In the packed assembly, the reaction is accelerated more prominently for substrates with higher hydrophobicity and is more tolerant of inhibitors (Figure 2).

When another enzyme xylose reductase was assembled in the packed state, a similar acceleration of the reaction in the packed state over the dispersed state was also observed (Figure 3). We propose that the entropic force of water increases local substrate concentration within the domain confined between enzyme surfaces, thus accelerating the reaction. Our finding offers a new insight on the efficiency of reaction by single type of enzyme in the packed state. The water-entropy effect increases as the enzyme structure becomes less flexible in the packed assembly. Therefore, a greater effect is expected within the spatially more constrained cellular compartments. Our system provides a reasonable model of enzymes in packed state; this would help in engineering artificial metabolic systems.

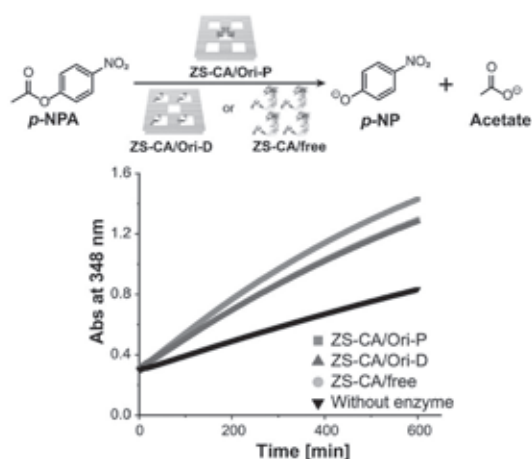


Fig. 2 Time-course profiles of the esterase reactions of ZS-CA (4 nM) on DNA scaffold with *p*-NPA was monitored at 348 nm.

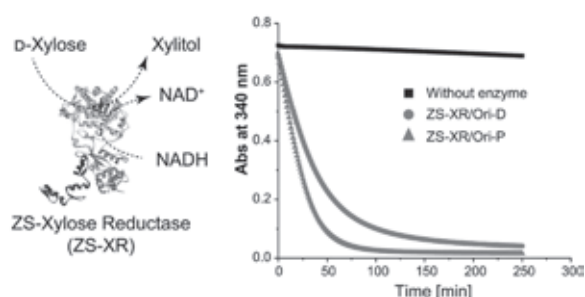


Fig. 3 Time course profiles for the reaction of ZS-XR on DNA scaffold monitored by the oxidation of NADH at 340 nm.

3. Evaluation of the role of the DNA surface for enhancing the activity of scaffolded enzymes

Enzymes have been widely applied in the chemical, medical and food industries. Immobilizing enzymes of interest on the surface of a carrier provides the simplest yet useful method for practical enzyme applications. Immobilized enzymes often display higher activity and stability than their free form; however, the exact mechanism for enhanced activity is still under debate. The catalytic enhancements of enzymes loaded on DNA nanostructures have been attributed to the characteristics provided by highly negative charges on the surface of the DNA scaffold, such as the modulation of the local pH near enzymes.

In this study, two enzymes with different pH preferences, xylose reductase (XR) and xylitol dehydrogenase (XDH), were individually assembled on a DNA scaffold through a modular adaptor. Catalytic enhancements were observed for both the scaffolded XR and XDH over the respective free enzyme. The different optimal pH profiles of XR (pH 6.0) and XDH (pH 8.0), the neutral or net negative charge of their substrates and cofactors indicated that neither the local pH

change nor the surface–substrate or –cofactor electrostatic attractive interactions accounted for the increase in the activities of the assembled enzymes. We also suggest that the improved stability or reduced adsorption of scaffolded enzymes alone is not the determining factor for enhancing the activity of enzymes on the DNA scaffold.

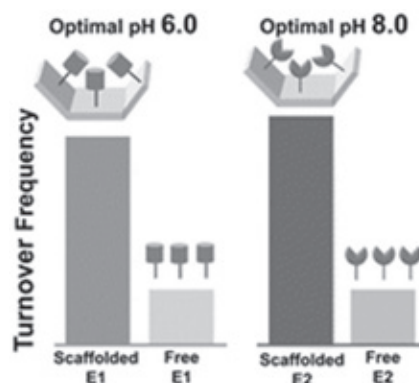


Fig. 4 Schematic representations and comparison of the activity of the free and scaffolded XR (left) and XDH (right).

4. RNA-Peptide Conjugation through an Efficient Covalent Bond Formation

RNA-peptide (RNP) complex is a good scaffold for construction of the receptor-based fluorescent sensors. We have reported the method for construction of RNP sensors that show the fluorescent intensity change upon binding the targeted molecule. We also have developed the method for formation of covalent linkage between RNA and peptide in order to improve the stability of RNP sensor. A representative method was applied for the formation of Schiff base or dihydroxymorpholino linkage between a dialdehyde group at the 3'-end of sugar-oxidized RNA and a hydrazide group introduced at the C-terminal of peptide subunit through a flexible peptide linker. In this study, we investigated effects of the solution pH and contribution of the RNA and peptide subunits to the conjugation reaction by using RNA and peptide mutants. The reaction yield reached 90% at a wide range of solution pH with reaction within 3 hours. The efficient reaction was mainly supported by the electrostatic interaction between the RNA subunit and the cationic peptide subunit of RNP scaffold.

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Collaboration Works

森井孝, Ghent University (ベルギー), クロスリンク反応性を内在する機能性生体高分子によるケミカルバイオロジーの開拓

森井孝, Rajendran Arivazhagan, Vanderbilt University School of Medicine (アメリカ), Topoisomerase 反応の可視化

森井孝, 仲野瞬, POSTECH (韓国), RNA 分子センサーの構築

森井孝, 中田栄司, Seoul National University (韓国), 特定空間に配置された酵素複合体の構築

森井孝, 中田栄司, 仲野瞬, Nanyang Technological University (シンガポール), RNP 分子認識の1分子計測

森井孝, 中田栄司, Rajendran Arivazhagan, Ewha Womans University (韓国), 小分子による酵素機構の解明

森井孝, 仲野瞬, POSTECH (韓国), 蛍光性バイオセンサーの構築

大垣英明, 森井孝, 片平正人, 野平俊之, モンゴル国立大学, インドネシア大学, フィリピン大学ディリマン校, ベトナム国家大学ハノイ校, ラオス国立大学, 王立ブノンペン大学, アジア新興国産天然資源を由来とする機能性物質創生のための高度分析研究拠点の形成

Financial Support

1. Grant-in-Aid for Scientific Research

森井孝, 基盤研究(A), 人工代謝経路を内包するナノ空間「複合触媒コンパートメント」の創出

中田栄司, 基盤研究(B), DNA ナノ構造体の階層的自己組織化による高効率な酵素連続反応場の構築

中田栄司, 新学術領域研究(研究領域提案型), 酵素間距離を制御する分子コンビナートをを用いた非天然化合物合成システムの創製

仲野瞬, 若手研究, 生成物解離を制御した RNA-ペプチド複合体リセプター酵素の創製

2. Others

森井孝, 科学技術振興機構 CREST, 細胞内環境測定多元同時センサーの開発

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Presentations

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