博士論文要約

論文題目

Construction of An Artificial Metabolic Channeling System on DNA origami

(DNA オリガミ上での人工代謝経路の構築)

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Background

Nature utilizes various degree of organization with the biological complexity. For instance, to perform the various enzymatic reactions and to process sustain life, the living cells require spatial organization of molecules. This is achieved via compartmentalization, the physical separation of biological reactions such as membrane bound organelles, bacterial microcompartments (BMCs), multienzyme complexes, and so on. Cells would have faced with many challenges when they lacked compartmentalization. For examples, some enzymes, *e.g.*, ribulose 1, 5-bisphosphate carboxylase oxygenase (RuBisCO), will suffer from slow turnover, which results in flux imbalances or bottlenecks in the pathways; the volatile intermediates will be lost by their diffusion through cellular membrane; the toxic intermediates, could be produced in the biosynthetic pathways that directly inhibit growth; the multiple competing cross-reactions can occur that resulting in reducing availability for any single pathway. To deal with these challenges, nature has involved compartmentalization strategies, e.g., the formation of large enzyme complexes, organelles, and BMCs, to spatially organize metabolism. The compartmentalization in the form of membrane-bound organelles is common in eukaryotes. For instance, lysosomes that contain an array of enzymes capable of breaking down all types of biomacromolecules (proteins, nucleic acids, carbonhydrates, and lipids) to function as the digestive system of the cell,

serving both to degrade material taken up from outside the cell and to digest obsolete components of the cell itself, or peroxisome encapsulates reactions that generate or consume hydrogen peroxide, a toxic intermediate from the breakdown of organic substrates in oxidative reactions. The formation of microcompartments in the prokaryotes partition the internal space of the bacterial cell for specialized functions. In cyanobacteria and other autotrophic prokaryotes, the carboxysomes encapsulate RuBisCO and carbonic anhydrase (CA), enzymes involved in the rate-limiting step of the Calvin cycle. These proteinaceous microcompartments are the primary carbon-concentrating mechanism in these bacteria. They are proposed to help overcome the slow turnover rate of RuBisCO by providing a high local concentration of CO_2 to enzyme. The compartmentalization are also formed through formation of multienzyme complexes, which directly link enzymes involved in a given pathway. Ideally, this results in substrate channeling as well as metabolic channeling, the process by which intermediates are directly transferred between the active sites of two enzymes that catalyze sequential reactions in the pathway. Substrate channeling prevents the loss of intermediates and minimizes competing cross-reactions.

Attempts to engineer and characterize the metabolic organizational systems will allow us to thoroughly understand cellular strategies of spatial organization. To mimick nature's mechanisms of metabolic channeling, a variety of methods have been developed, *e.g.*, immobilized enzyme systems, fusion proteins, post-translational assembly, synthetic protein scaffolds, and discussed in depth. However, all of these methods have a limitation for studying on the effect of spatial organization, *e.g.*, position, orientation, and enzyme ratio, on enzymatic activity in multienzyme systems. By taking advantages of DNA as generic material, the two-, three-dimensional DNA nanostructures have been constructed. It has opened a new way to spatially organize biomolecules with nanometre precision on DNA nanostructures with molecular binding sites at designated positions. This method is very promising to exploit mechanisms of metabolic channeling depend on the distance and ratio between the enzymes on DNA nanostructures.

Purpose of research

To understand the mechanism of cofactor recycling and effects of local cofactors or substrates concentrations to enzymatic activities in the metabolic pathway, I have constructed functional artificial metabolic channeling systems based on xylose metabolic pathway. With this new model system, enzymatic activities by providing high local concentration of cofactors to enzymes on DNA origami scaffolds are investigated.

Contents of research

Chapter 1 is general introduction that describes the background of research carried out in this thesis. In Chapters 2 and 3, I have focused on development of a homodimeric adaptor to locate a functional protein dimer on DNA origami. Proteins are particularly interesting class of molecules to assemble on the specific addresses of DNA nanostructures because of their huge functional variations. Methods to attach proteins at specific locations on DNA scaffolds have been reported, however, almost all of these methods rely on the post-modification of proteins by oligo DNA prior to their assembly, which tends to reduce the functional activity of proteins. Therefore, a method that is fully based on a protein component would accelerate the specific assembly of proteins in the functional form on the DNA nanoarchitectures. The sequence-specific DNA binding protein acts as an ideal adaptor for locating a protein of interest at the specific address of DNA nanoarchitectures. I have utilized a monomeric zinc finger protein, a well-studied class of the sequence-specific DNA binding proteins, as an adaptor for monomeric protein to target at specific location on DNA origami and demonstrated that the adaptor-fused auto-fluorescent proteins were assembled in the functional form at specific locations on a DNA origami scaffold, termed as molecular switchboard. Development of various types of adaptors with distinct sequence selectivity enables orthogonal location of various adaptor-fused proteins on DNA origami at specific positions. In this chapter, I have applied a basic-leucine zipper (bZIP) class of protein GCN4 as a new adaptor to expand both the range of target DNA sequences and the variety of proteins applicable for the adaptor strategy.

In Chapter 4, development of a new class of covalent cross-linking adaptors that realize rapid and efficient immobilization of protein of interest (POI) on DNA origami by combination of the DNA binding protein adaptor (zif268) and the self-ligating protein tag (SNAP-tag) is described. Formation of a covalent linkage between DNA and POI is a promising strategy to overcome the reversibile nature of the resulting complex which caused difficulty in locating the adaptor-fused POI in a stoiciometric manner and in preserving the located POI to be intact during the assay procedures.

In Chapters 5 and 6, inspired of mother principle in nature, we concentrate on the demonstration of an artificial metabolic channeling on DNA origami and a model of enhancement enzymatic activity on DNA origami by providing high local cofactors concentration to enzymes. By taking advantage of protein adaptor derivatives (zif268 and GCN4), two functional enzymes (xylose reductase (R276H) and xylitol dehyrogenase (XDH)) were located on DNA origami at specific position, the enzymatic activity of the artificial metabolic channeling was controlled in reorganization of two enzymes on DNA origami by means of changing interenzyme distances and ratio of binding sites for both enzymes. The immobilization of cofactor receptors (RNP) surrounding enzymes (xylitol dehydrogenase or luciferase) on DNA origami results in enhancement of enzymatic activities. This system offer a great opportunity for bottom-up nanofabrication of bio-tool with desired biochemical pathway.

Significance of research

This study provides a new adaptor to locate a protein dimer, one of the commonest forms of enzymes, on the DNA origami scaffold based on a homodimeric basic-lucine zipper protein GCN4. As a evidence for this adaptor, a GCN4-fused homodimeric enzyme (GCN4-XDH) showed even higher activity than the wild type enzyme, and exhibited avid reactivity when assembled at the specific site of DNA origami. Along with monomeric adaptor (zif268), a new method, which did not reduce the functional activity of enzymes comparing to other methods, was developed to arrange orthogonally enzymes on DNA origami with nanometer-scale precision. The combination of the DNA binding protein

adaptor (zif268) and the self-ligating protein tag (SNAP-tag) give us a new class of covalent cross-linking adaptor to realize rapid and efficient immobilization of protein of interest (POI) on DNA origami. By taking advantage of these protein adaptors, a model of real functional metabolic channeling was demonstrated on DNA origami scaffold with controllable distances and ratio between enzymes. This system will open a new way to study artificial protein networks. On the other hand, another novel model for enhancement of enzymatic activity by providing high local cofactor concentration to enzyme was demonstrated based on coassembly of cofactor receptors and enzymes on DNA origami. This offer a great opportunity for bottom-up nanofabrication of bio-tool with desired biochemical pathway. To the best of our knowledge, both of models were demonstrated on DNA origami scaffold for the first time.

Future perspectives

Attempts to mimic the metabolic network inside the cell not only expands our knowledge of cellular metabolic systems but also opens a possibility to create nano-reactors that have broad range of applications from diagnostics to the production of high-value chemicals. The successes in demonstration of model for an artificial metabolic channeling based on coassembly of two enzymes (e.g., xylose reductase and xylitol dehydrogeanse) on DNA origami offer a great opportunity for fully study on numerous other multienzyme complexes in living cells. For example, the organization of topological self-assembly of photosynthetic reaction center on the DNA origami scaffold represent exciting future opportunities. The combination of self-assembled DNA nanostructure with functional biomolecules may result in the development of potential ways to regulate cellular response. It may be feasible to construct artificial intra- or extra-cellular nanomatrices that are designed to influence modulate biological pathways.