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Detection of conformationally changed MBP using intramolecular FRET

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ABSTRACT

The principal objective of this study was to explore protein conformational changes using fluorescence resonance energy transfer (FRET) technology. Maltose binding protein (MBP) was adopted as a target model, due to its well-characterized structure and ligand specificity. To the best of our knowledge, this is the first report to provide information regarding the biological distance between the two lobes of MBP upon maltose binding. For the FRET pair, ECFP and EYFP were used as the donor and the acceptor, and were linked genetically to the C-terminal and N-terminal regions of MBP (ECFP:MBP:EYFP), respectively. After the FRET reaction, maltose-treated MBP was shown to exhibit a considerable energy transfer (FRET efficiency (E) = ~0.11, Distance (D) = ~6.93 nm) at the ensemble level, which was regarded as reflective of the increase in donor quenching and the upshift in acceptor emission intensity, thereby suggesting that the donor and the acceptor had been brought close together as the result of structural alterations in MBP. However, upon glucose treatment, no FRET phenomenon was detected, thereby implying the specificity of interaction between MBP and maltose. The *in vitro* FRET results were also confirmed via the acceptor photobleaching method. Therefore, our data showed that maltose-stimulated conformational changes of MBP could be measured by FRET, thereby providing biological information, including the FRET efficiency and the intramolecular distance.

Fluorescence resonance energy transfer, referred to more commonly by the acronym FRET, is one of the most versatile technologies among all currently available fluorescence methods [1,2]. FRET can occur when an excited donor fluorophore transfers energy to an acceptor, thereby quenching the donor emission and increasing the emission of the acceptor [2,3]. Several factors affect FRET efficiency: (1) the distance between the donor and acceptor fluorophores (1–10 nm), (2) the special overlap between the spectrum of donor emission and the absorption spectrum of the acceptor (30%), and (3) the desirable orientation of the donor and acceptor fluorophores' dipoles [4,5]. After the basic theory of FRET was established by Förster [6], the applications of FRET technology for biological sensing—which include nucleic acid hybridizations [7,8], protein–protein interactions [9,10], protein–DNA interactions [11,12], and protein conformational changes [13,14]—have been expanded significantly, as the result of its rapid signal response time, which is achieved without any sacrifice of sensitivity.

Considering the benefits of this particular fluorescence protocol, the principal objective of this study was to detect protein conformational changes using FRET technology. Here, maltose binding protein (MBP) as a model biomolecule was employed in order to evaluate structural transition, owing to its well-characterized structure and ligand specificity [15,16]. Indeed, MBP has been previously studied by a number of researchers as a model protein for structural transition [17,18]. Because two fluorophores are basically required for a FRET assay, enhanced cyan fluorescent protein (ECFP) as the donor fluorophore, and enhanced yellow fluorescent protein (EYFP) as the acceptor fluorophore were genetically fused to the C- and N-termini of MBP (ECFP:MBP:EYFP), respectively. After maltose treatment, the quenching of emission from the donor or increase in acceptor emission intensity was assessed. Consequently, MBP that was conformationally changed in response to

Abbreviations: FRET, fluorescence resonance energy transfer; MBP, maltose binding protein; ECFP, enhanced cyan fluorescent protein; EYFP, enhanced yellow fluorescent protein; IPTG, isopropyl β-D-thiogalactopyranoside; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis

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maltose treatment evidenced a considerable FRET efficiency of 0.11, thereby indicating that the donor and the acceptor were located close together. This FRET efficiency indicates that the conformationally changed MBP was brought into a closed configuration (or maltose-bound form) as the result of the spatial proximity between the two lobes of MBP upon maltose binding. By way of contrast, FRET has seldom been observed with glucose stimulation as a negative control, thereby indicating ligand-specific structural alteration. The results of this study clearly demonstrated that FRET technology might prove a useful tool for the monitoring of maltose-induced structural alterations in MBP, thereby potentially shedding new light on the FRET-based monitoring of intramolecular conformation transitions.

Materials and methods

Construction of the pECFP:MBP:CYFP plasmid. In order to clone the ECFP:MBP:EYFP fusion gene, the full-length gene encoding for ECFP (719 amino acids) was initially amplified with the 5′ primer (GGAATTCCATATGGTGAGCAAGGGCGAG) and the 3′ primer (GAAGATCTCTTGTACAGCTCGTC) via polymerase chain reaction (PCR). The 5′ and 3′ termini were designed to contain the *Nde*I and *Bgl*II restriction enzyme cleavage sites, respectively. In order to generate the EYFP, the full-length gene encoding for the EYFP (719 amino acids) was amplified via PCR with the 5′ primer (CCCAAGCTTATGGTGAGCAAGGGCGAG) and the 3′ primer (CCGCTCGAGCTTGTACAGCTCGTC) with the *Hin*dIII and *Xho*I restriction enzyme cleavage sites, respectively. The MBP gene was subsequently PCR-amplified using the 5′ primer (ACTGGATCCATGAAAACTGAAGAAGGT) and the 3′ primer (ACTAAGCTTAGTCTGCGCGTCTTTCAG) with the *Bam*HI and *Hin*dIII restriction enzyme cleavage sites, respectively. The PCR products were then purified using a DNA purification kit (Qiagen), and digested with the indicated restriction enzymes. The resultant DNA fragments were subsequently ligated with the pET 21a vector, using a ligation kit (Takara, Japan) (pECFP:MBP:EYFP), and cloned into the pET 21a vector using the *Nde*I and *Xho*I sites, thereby generating the ECFP:MBP:EYFP in-frame fusion. The ECFP:MBP:EYFP fusion gene was confirmed via DNA sequencing.

Expression of the chimeric ECFP:MBP:CYFP protein. The pECFP:MBP:EYFP construct was transformed into *E. coli* DH5α. The ampicillin-resistant colonies were screened via the DNA isolation of 3 ml overnight cultures, followed by restriction mapping. Plasmid DNA was prepared and purified using a QIAprep spin miniprep kit (Qiagen, Germany). The plasmids were then transferred to the expression host, *E. coli* BL21 (DE3) (Stratagene, CA), and then plated onto LB plates. A single colony from a fresh plate was picked and grown at 37 °C in 3 ml of Luria–Bertani (LB) broth containing 50 mg/ml of ampicillin, to an OD_{600} of 0.6. This was then inoculated into 100 ml of LB broth containing ampicillin. The cells were grown

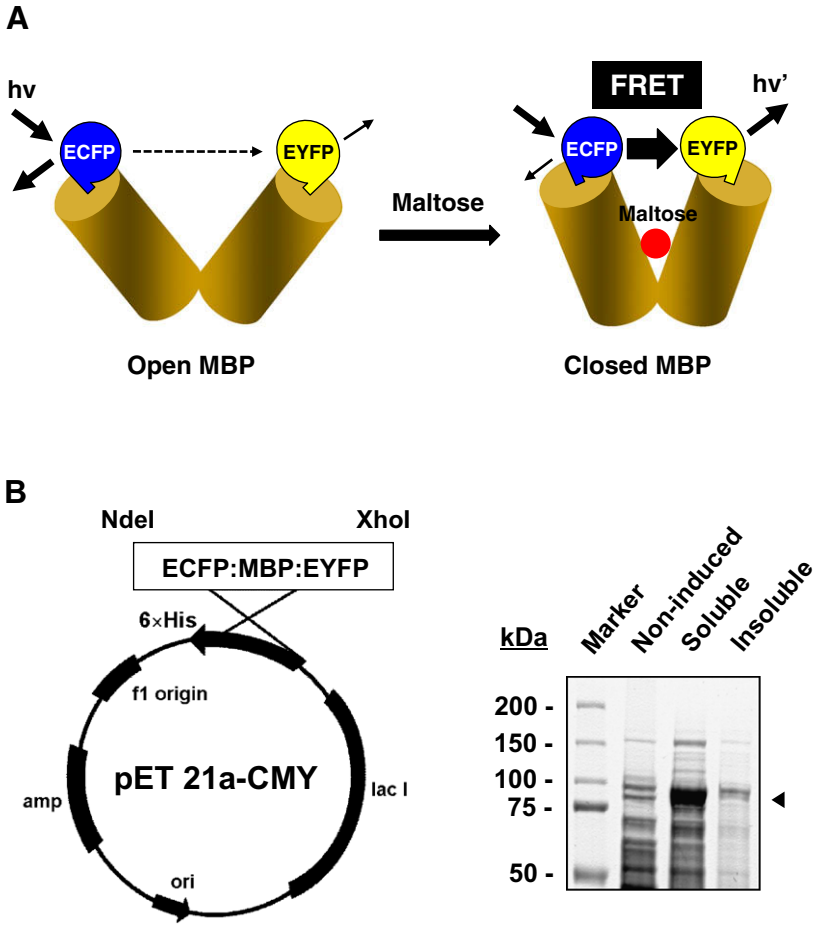

Fig. 1. Schematic diagram of FRET for analyzing maltose-induced conformational change in MBP. (A) The ECFP donor and the EYFP acceptor were genetically attached to the C- and N-termini of MBP, respectively. Upon the addition of maltose, the donor emission intensity is expected to decrease, and the acceptor emission will subsequently increase. (B) Construction of the pET 21a-CMY vector (pECFP:MBP:EYFP) for expression of ECFP:MBP:$EYFP_{6\times His}$. amp, ampicillin-resistant gene; ori, origin of replication. (C) SDS–PAGE analysis of chimeric ECFP:MBP:EYFP protein. Lane 1, protein marker; Lane 2, total cell lysates of non-induced BL21 (DE3); lane 3, soluble fraction of IPTG-induced BL21 (DE3); lane 4, insoluble fraction. The arrow indicates the expressed ECFP:MBP:$EYFP_{6\times His}$ protein.