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Advantages of substituting bioluminescence for fluorescence in a resonance energy transfer-based periplasmic binding protein biosensor

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ABSTRACT

A genetically encoded maltose biosensor was constructed, comprising maltose binding protein (MBP) flanked by a green fluorescent protein (GFP^2) at the N-terminus and a Renilla luciferase variant (RLuc2) at the C-terminus. This Bioluminescence resonance energy transfer^2 ($BRET^2$) system showed a 30% increase in the BRET ratio upon maltose binding, compared with a 10% increase with an equivalent fluorescence resonance energy transfer (FRET) biosensor. $BRET^2$ provides a better matched Förster distance to the known separation of the N and C termini of MBP than FRET. The sensor responded to maltose and maltotriose and the response was completely abolished by introduction of a single point mutation in the $BRET^2$ tagged MBP protein. The half maximal effective concentration (EC_{50}) was 0.37 μM for maltose and the response was linear over almost three log units ranging from 10 nM to 3.16 μM maltose for the $BRET^2$ system compared to an EC_{50} of 2.3 μM and a linear response ranging from 0.3 μM to 21.1 μM for the equivalent FRET-based biosensor. The biosensor's estimate of maltose in beer matched that of a commercial enzyme-linked assay but was quicker and more precise, demonstrating its applicability to real-world samples. A similar $BRET^2$-based transduction scheme approach would likely be applicable to other binding proteins that have a "venus-fly-trap" mechanism.

1. Introduction

Periplasmic binding proteins (PBPs) are a large and diverse family of soluble proteins found in bacteria. PBPs bind a wide range of chemical species, including carbohydrates, amino acids, neurotransmitters, metal and other ions (Medintz and Deschamps, 2006). Although PBPs are unrelated at the primary sequence level they all undergo a large ligand-induced conformational rearrangement commonly referred to as the 'venus-fly-trap' mechanism (Sharff et al., 1993, 1992; Spurlino et al., 1991). Due to the potentially large pool of analytes that can be recognised by members of the PBP superfamily, they have been extensively exploited as biological recognition elements for biosensors in a wide range of application areas including security, food and drink quality control, environmental monitoring and health-care (Dwyer and Hellinga, 2004). In this study, we set out to improve the sensitivity and dynamic range of a model PBP based biosensor. A biosensor is an analytical device which combines a biological recognition element, such as a periplasmic binding protein, to a transducer element. The use of bioluminescence instead of fluorescence as the biosensor transduction element of the PBP based biosensor reduces the cost and complexity of equipment needed to read the signal and markedly lowers the limits of detection compared with any comparable, genetically encoded biosensor.

Fehr et al. (2002) developed a genetically encoded FRET based maltose biosensor with cyan fluorescent protein (CFP) at the N-terminus and yellow fluorescent protein (YFP) at the C-terminus of maltose binding protein (MBP). Maltose induced a conformational rearrangement in MBP bringing the CFP and YFP closer and increasing the energy transfer rate from the CFP donor to the YFP acceptor. However, the output signal, the change in FRET ratio, has a narrow dynamic range, necessitating the use of sophisticated reading equipment and spectral deconvolution.

The narrow dynamic range of the FRET ratio response is due to the low spectral separation between the donor and acceptor emission spectra, which is inherent to the low Stokes shift of both FRET components. The spectral separation between donor and acceptor emission peaks is doubled by replacing the fluorescent donor protein with a luciferase, converting the transduction principle to bioluminescence resonance energy transfer (BRET) (Pfleger and Eidne, 2006; Pfleger et al., 2006). The $BRET^2$ system consists of a Renilla luciferase (RLuc) with coelenterazine 400a (Clz400a) as substrate for the donor system and a modified green fluorescent protein (GFP^2) acceptor. We previously showed that BRET is more sensitive than FRET for measuring proteolytic cleavage (Dacres et al., 2009a, b, 2012a) and $BRET^2$ has a larger Förster distance than classical FRET (Dacres et al., 2010).

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This property made BRET^2 more suitable than FRET for transducing ligand-induced activation of GPCRs (Dacres et al., 2011) because the 6.8 nm separation of the BRET^2 donor and acceptor is a good match to its Förster distance. We noted that the measured distance of 6.9 nm between the FRET tagged N and C termini of MBP of (Park et al., 2009) is almost identical to the apparent distance between the tags on the aforementioned GPCR (Dacres et al., 2011). BRET^2 is more sensitive to movements in this range than classical FRET (Fig. S-1, Supporting information).

We therefore predicted that substituting BRET^2 donor and acceptor pairs for FRET reporters in a PBP-based sensor (Fig. 1) would enable a larger dynamic range in the RET output ratio with ensuing advantages for sensitivity and precision. We chose MBP as an initial test of this prediction because it is a well-characterised member of the PBP superfamily and is potentially representative of many other PBPs. We report here the development of a novel BRET based biosensor for monitoring ligand binding by periplasmic binding proteins, with greatly enhanced sensitivity over earlier methods.

2. Materials and methods

2.1. Construction BRET proteins

The BRET^2 fusion partners RLuc2 (C124A/M185V), RLuc8 (A55T/C14A/S130A/K136R/A143M/M185V/M253L/S287L) and GFP^2 were amplified and restriction cloned into a series of BRET fusion proteins containing maltose binding protein (MBP). The W140A mutation was introduced into pRSET GFP^2-MBP-RLuc2 using site-directed mutagenesis (Stratagene). Standard molecular biology techniques were used with primers shown in Table S-1 (Supporting information). All clones were sequenced to confirm their integrity and orientation.

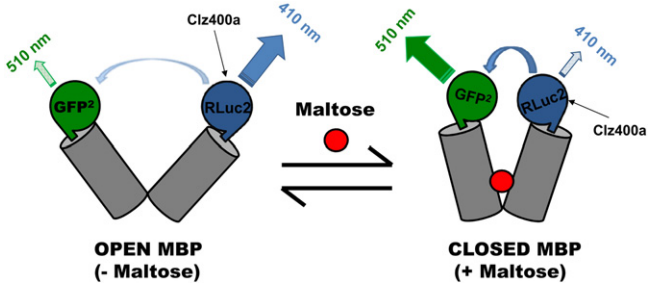

Fig. 1. BRET^2 transduction principle for the GFP^2-MBP-RLuc2 biosensor incorporating green fluorescent protein (GFP^2), MBP and a variant Renilla luciferase (RLuc2). Maltose binding causes a conformational change in the biosensor bringing the BRET donor and acceptor closer and increasing the efficiency of energy transfer from RLuc2 to GFP^2. Clz400a = Coelenterazine 400a.

2.2. Expression and purification of RET proteins

Constructs were transformed into electrocompetent BL21 (DE3) cells (Novagen). At least three independent colonies were selected for each construct and used to perform biological replicates. Cultures were grown up and lysed using a homogeniser (Avestin emulsiflex C3 (ATA Scientific)). The BRET^2 constructs were affinity-purified over TALON™ Superflow Metal Affinity Resin (Clontech Laboratories, Inc.) and their purity was confirmed using SDS-polyacrylamide gel electrophoresis (Fig. S-2 in Supporting information). 1 μM purified protein was used for all BRET assays unless otherwise stated.

2.3. BRET^2 detection

Spectral scans were recorded with a SpectraMax M2 plate-reading spectrofluorimeter (Molecular Devices). Simultaneous dual emission BRET measurements were carried out with a POLARstar OPTIMA microplate reader (BMG LabTech). BRET^2 measurements used the BRET^2 emission filter set comprising RLuc/CLZ400a emission filter (410 nm bandpass, 80 nm) and the GFP^2 emission filter (515 nm bandpass, 30 nm). BRET^2 ratios were calculated as ratios of integrated acceptor emission channel intensity to integrated donor emission channel intensity.

For full experimental details, see supplementary materials and methods (Supporting information).

3. Results and discussion

3.1. BRET comparisons

3.1.1. BRET intensity

Based on recent findings (Dacres et al., 2010) BRET^2 provides the best matched Förster distance to the known separation of the N and C termini of MBP (Fig. S-1, Supplementary information) (Park et al., 2009). However, BRET^2 has a low quantum yield and rapid decay kinetics when used with the Clz400a substrate (Pfleger and Eidne, 2006). However, it was recently shown that the sensitivity of the BRET^2 assay can be significantly improved by use of RLuc mutants (e.g., RLuc2 and RLuc8) with improved quantum yields and stability (De et al., 2007; Loening et al., 2006). We compared the BRET^2 based MBP sensors incorporating native RLuc, RLuc2 and RLuc8 (Fig. 2).

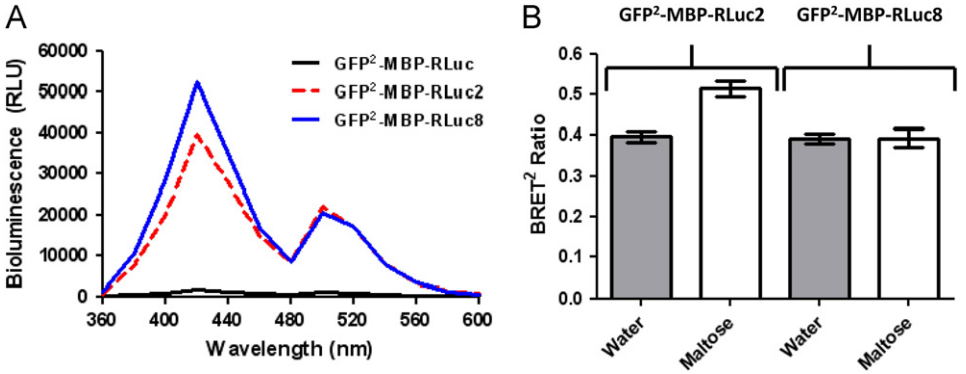

Fig. 2. (A) Bioluminescence spectra upon the addition of 16.7 μM coelenterazine 400a substrate to 1 μM BRET fusion proteins GFP^2–MBP–RLuc, GFP^2–MBP–RLuc2 and GFP^2–MBP–RLuc8. 20 nm intervals were used. (B) BRET^2 ratio upon the addition of 16.7 μM coelenterazine 400a substrate to 1 μM purified BRET fusion protein GFP^2–MBP–RLuc2 or GFP^2–MBP–RLuc8 following addition of 10 μl water (grey bars) or 10 μl maltose solution in water (final concentration 0.1 mM).