2014 年度修士論文要旨

Development of an intracellular inorganic carbon nanosensor based upon Förster resonance energy transfer (FRET)

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[Introduction]

Inorganic carbons such as CO2 and HCO3- have the important role in photosynthesis and otherfundamental metabolisms for many living organisms. There are several methods that have been used formeasurement of inorganic carbon like gas chromatography (GC), infrared-gas analyzer (IRGA), membraneinletmass spectrometry (MIMS), and CO2 electrode. However, these known measurement methods are unableto measure the localized intracellular concentration of HCO3- and/or CO2. The objective of this study is development an intracellular inorganic carbon nanosensor based on Förster resonance energy transfer (FRET).FRET is a phenomenon of distant-dependent energy transfer between fluorophores, resulting in the enhancedacceptor fluorescence intensity with concomitant reduction of donor fluorescence intensity. Cyanobacterial cytoplasmic membrane protein A (CmpA) of Synechococcus elongatus PCC 7942, which is known to bind specifically to HCO3- and changes its structure upon binding HCO3- (1) was used as a core nanosensor protein by fusing with a donor/acceptor fluorophore pair, CFP/Venus.

[Experimental]

CmpA based nanosensor candidates were constructed and transformed into E. coli. These constructs were combined with the visual advantages of Green fluorescent protein (GFP) variants to design a nanosensor for the determination of DIC concentration in vitro. The mutations in bicarbonate binding site of truncated CmpA from Synechococcus elongatus PCC7942 (55 to 416 amino acid relative to the initial methionine) were introduced to CmpA by PCR-based-site-directed mutagenesis, which contains CFP and circular-permuted Venus (cp229Venus). As well as, the truncated CmpA from Synechocystis were inserted between Venus and CFP. These constructs were transformed into E. coli BL21 (DE3). Protein expression was induced by IPTG at 37°C for 4 hours or 20°C for 20 hours, and cells were disrupted by sonication. The protein expression was checked by SDS-PAGE and subsequent western blotting with Anti-GFP IgG as a primary antibody. After the designed protein was found in the soluble fraction, protein was purified by ProfanityTM IMAC Resin (Biorad). FRET under various DIC concentrations from 15 μM to 150 μM was measured by fluorescence spectrophotometer and GC, respectively. The measurement condition of spectrophotometer of CFP excitation and YFP excitation were taken at 400 nm and 480 nm, respectively.

[Results and discussion]

FRET-based nanosensors are frequently used for the analysis of molecular processes within living cells (2). In the present study, a series genetically encoded bicarbonate sensors was present based upon FRET. The CmpA-based nanosensor candidates were produced in E.coli

protein expression system and the FRET characteristics of purified nanosensor candidates were done. The spectral measurement of some truncations of the end N/C termini of CmpA showed a decreased of CFP and Venus intensity while [HCO3-] was increased. If FRET is occurred, the isosbestic point should be appeared in the typical spectra upon change of DIC concentration. The normalized spectra of all CmpA-based nanosensor showed that the changes of CFP and YFP intensities were not significant. FRET efficiency of CmpA D6, CmpA G6, and CmpA A6 were decreased during increasing the [HCO3-] indicated the negative correlation of FRET ratio to DIC. In contrast, CmpA WT revealed a positive correlation between FRET efficiency and [HCO3-], indicating that CmpA proteins can potentially be a basal nanosensor body. Several site-directed mutants like CmpA N149D, CmpA E267Q did not show changed FRET efficiency depending on the increasing HCO3- concentration, the similar results showed in CmpA G6 and CmpA A8. In order to make the larger FRET dynamic range, Ca2+ was added in purification steps as this metal ion is essential for the HCO3- binding site of CmpA(3). The results showed the slightly enhanced changes in the FRET ratio in response to increase in [DIC].

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(2) Salonikidis, P.S. et al., Biophysj. 95, 5412-5423 (2008)
(3) Koropaktin, N.M. et al., J. Biol. Chem. 282, 2606-2614 (2006)