



Title	Development of coelenterazine derivatives for monitoring of biological phenomena
Author(s)	Lindberg, Eric
Citation	大阪大学, 2013, 博士論文
Version Type	VoR
URL	https://doi.org/10.18910/34469
rights	
Note	

The University of Osaka Institutional Knowledge Archive : OUKA

<https://ir.library.osaka-u.ac.jp/>

The University of Osaka

Abstract of Thesis

Name (Eric Jorgen Lindberg)	
Title	Development of coelenterazine derivatives for monitoring of biological phenomena (生命科学現象を可視化するセレンテラジン誘導体の開発)
<p>The general aim of this thesis is the modification of coelenterazine to generate new bioluminogenic tools for chemical biology. The development of the first cell-membrane impermeable coelenterazine derivative (CoelPhos) is described. CoelPhos was constructed by alkylation of coelenterazine with a linker containing a terminal anionic phosphonate moiety. Bioluminescence activity of CoelPhos with <i>Gaussia</i> luciferase (GLuc) showed a significantly higher activity in comparison with <i>Renilla</i> luciferase. In imaging studies with living cells, outer membrane bound GLuc was clearly imaged with CoelPhos. On the other hand no signal could be detected with intracellularly localized GLuc, demonstrating the impermeability of this novel coelenterate substrate derivative. CoelPhos has potential utility as a new bioluminogenic tool for monitoring of dynamic fusion events at the cell surface interface. The second part of the research was focused on developing caged coelenterazine compounds as dual-substrates. The approach addresses the instability of coelenterazine by introducing caging groups to block the auto-oxidation of coelenterazine. Two bioluminogenic caged coelenterazine derivatives (bGalCoel and bGalNoCoel) were designed and synthesized to detect β-galactosidase activity and expression by means of bioluminescence imaging. Both probes contain β-galactosidase cleavable caging groups at the carbonyl group of the imidazopyrazinone moiety. One of the probes in particular, bGalNoCoel, displayed a fast cleavage profile, high stability, and high specificity for β-galactosidase over other glycoside hydrolases. bGalNoCoel could detect β-galactosidase activity in living HEK-293T cell cultures that expressed a mutant GLuc. It was determined that coelenterazine readily diffuses in and out of cells after uncaging by β-galactosidase. It was showed that this new caged coelenterazine derivative, bGalNoCoel, could function as a dual-enzyme substrate and detect enzyme activity across two separate cell populations. In addition a coelenterazine-boronic acid conjugate was also developed (Coel-BeBA), which could be used to detect endogenously generated hydrogen peroxide in living RAW264 cells expressing GLucM23in the ER. Thus these coelenterazine derivatives will help to expand the bioluminescence toolbox for chemical biology.</p>	

論文審査の結果の要旨及び担当者

氏 名 (LINDBERG ERIC JORGEN)			
	(職)	氏	名
論文審査担当者	主 査	教 授	菊地 和也
	副 査	教 授	福住 俊一
	副 査	教 授	伊東 忍
	副 査	教 授	金谷 茂則
	副 査	教 授	高井 義造
	副 査	教 授	渡部 平司
	副 査	教 授	兼松 泰男

論文審査の結果の要旨

This dissertation describes the development of bioluminogenic tools for monitoring of biological phenomena via bioluminescence based on the modification of the substrate coelenterazine. With original synthetic methods coelenterazine could be customized for various applications including: monitoring of membrane trafficking events, detection of enzyme activity or bioactive small molecules. The principal results of this dissertation are summarized below.

Chapter 1 describes the development of cell-membrane impermeable coelenterazine derivatives as a potential bioluminogenic tool for monitoring of dynamic fusion events at the cell surface interface. By installing a terminal phosphonic linker at the 2-position of coelenterazine, CoelPhos was synthesized. Results showed that CoelPhos has adequate bioluminescence activity with Gaussia luciferase and decreased membrane permeability.

Chapter 2 focuses on activation and stabilizing of the coelenterazine substrate by blocking auto-oxidation with groups cleavable by enzymatic or bioactive molecules.

Chapter 2.1 describes the development of activatable coelenterazine derivatives for monitoring of β -galactosidase activity. The probe bGalNoCoel showed fast cleavage profile, high stability, and high specificity for β -galactosidase. In addition it was demonstrated that bGalNoCoel could function as a dual substrate to monitor enzyme activity across two distinct cell populations.

Chapter 2.2 describes the development of hydrogen peroxide activatable coelenterazine derivatives. The probe CoelBeBA was constructed by conjugating an aryl boronic acid to coelenterazine. in vitro experiments demonstrate the ability of this probe to detect endogenous hydrogen peroxide in living cells.

As stated above, this dissertation describes for the first time how coelenterazine can be modified for investigating specific biological events such as exocytosis at the cell-surface interface, detection of β -galactosidase activity, and detection of hydrogen peroxide in living cells. It is expected that this conceived strategy will allow for the development of many specific probes based on coelenterazine, leading to significant expansion of the bioluminescence toolbox for various applications in the life sciences. Thus this dissertation has the necessary merit to be considered a doctoral dissertation.