

Application Note

Detection of Light-Induced cAMP Production in Living Cells

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Summary

With optogenetic methods, signal transduction molecules can be activated using light and the affected signaling cascades can be analyzed in vivo. Signal transduction molecules, such as the photoactivatable adenylate cyclase bPAC of the sulfur bacterium *Beggiatoa sp.* produce the second messenger cyclical adenosine monophosphate (cAMP). The enzymatic formation of cAMP—and thereby the light-induced adenylate cyclase activity—can be detected with the GloSensor™ cAMP Assay using a luminescence signal.

Signal Transduction Studies with Optogenetic Tools

Optogenetics is a combination of methods from genetic engineering and optics: Photosensitive proteins are constructed using molecular biological methods (1). These modified proteins enable light-dependent monitoring of specific signal transduction paths (2,3). Optogenetic technologies enable a “more targeted activation” of specific signaling networks than is possible with current pharmacological methods. This is because a common disadvantage of traditional ligand and agonist applications is that more than one receptor group is activated. Where this occurs, the cellular response cannot be traced back specifically to one signal transduction cascade.

Modulation of cAMP Concentration Using the Adenylate Cyclase bPAC

In eukaryotic cells, information is communicated to the cell interior by means of specific signal transduction cascades. Numerous signaling molecules are involved in signal transmission. cAMP stimulates effector proteins as a second messenger and can cause varied effects in the cells.

One optimal optogenetic tool for investigating cAMP-dependent signaling pathways is the adenylate cyclase bPAC of the sulfur bacterium *Beggiatoa sp.* This bacterial bPAC contains a photosensitive sensor of blue-light using FAD (BLUF) domain and a C-terminal catalytic domain of a class III adenylate cyclase (4). After photoactivation, the enzyme bPAC converts cellular ATP into cAMP. Intracellular cAMP concentrations can therefore be regulated dynamically using light. The enzymatic activity of bPAC

was confirmed in *E. coli*, *Drosophila*, *Xenopus* oocytes and *Rodentia* neurons and can be increased by up to 300 times using suitable illumination (4). The absorption maximum is 453 nm in the light-adapted state (4). Detection in the cell complex is therefore easily possible using a long-wave fluorescence marker. With 350 amino acids, bPAC is suitable for vector expression systems with a finite capacity, such as viral vectors.

Direct Detection of Relative cAMP Concentration in HEK 293C Cells

The enzymatic formation of cAMP can be detected directly using the GloSensor™ cAMP Assay (Promega). The luciferase of the firefly *Photinus pyralis* was modified for this assay at the molecular genetic level to contain a cAMP binding site (5). When cAMP binds to luciferase, the luciferase is activated by a conformational change and emits light. A rise in cAMP concentration correlates with an increased luminescence signal, which can be quantified with any standard plate reader setup (5). To examine the photoactivatability of bPAC, HEK 293 cells were co-transfected with the luciferase-coding 22F plasmids and a bPAC-coding plasmid.

Detection of bPAC Light Dependence

The photoactivatable adenylate cyclase bPAC converts cellular ATP into cAMP at a wavelength of 450 nm. With repeated light stimulation, the luminescence signal measured accumulates (Figure 1). As relative cAMP concentrations and luminescence correlate, the enzymatic activity of the adenylate cyclase examined can be stimulated using light. This confirms two things: the photoactivatability of bPAC and the practical applicability of GloSensor™ technology for detecting bPAC activity by means of the increase in cAMP concentration.

Conclusion

For the adenylate cyclase bPAC, it was possible to use the GloSensor™ cAMP Assay to confirm the dependence of bPAC activity on the light stimulus.

The GloSensor™ cAMP Assay is a user-friendly, luminescence-based method for cAMP-dependent activity detection, and there-

fore offers an alternative to electrophysiological detection methods, which are currently preferred by optogeneticists. The assay permits the quantification of cAMP in real time under non-lytic conditions in cell culture. Relative cAMP concentrations can be measured in living cells with high temporal resolution (5).

References

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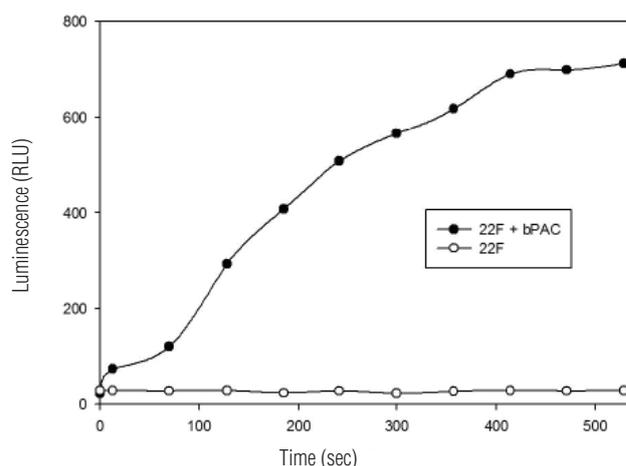


Figure 1. Stimulation of the adenylate cyclase bPAC at 450nm. Quantification was carried out on a plate reader (PerkinElmer) using the 96-well microtiter plate version of the GloSensor™ cAMP Assay. The representative quantification of a well containing HEK 293 cells, 48 hours after (co-)transfection of the bPAC-coding vector with the 22F plasmid (black) or the 22F control plasmid alone (white) is shown. Measurement process: Luminescence counting for three seconds after short-wave light stimulus (every ten seconds with a wavelength of 450nm). The measurement latency is subject to technical factors determined by the setup.