Photocontrol of Small G-protein Ras using regulatory factor GAP modified with photochromic molecular nanodevices.

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1. Introduction

The small GTPase Ras is a central regulator of cellular signal transduction processes leading to transcription, cell cycle progression, growth, migration, cytoskeletal changes, apoptosis, cell survival, and senescence, and functions as a molecular nanomachine. The switching mechanism is well-known at the molecular level and is considered as an intracellular signaling nanomachine. It is known that Ras is activated in the GTP bound state and inactivated in the GDP bound state, i.e., it is downregulated by its GTPase activity. Ras can transduce a signal through its effectors, which include Raf kinases, phosphoinositide 3-kinases, Ral guanine nucleotide dissociation stimulator, and phosphatases C, by the conformational change brought on by GTP binding. The conversion of GDP to GTP state and GTP to GDP state are mediated by the factors guanine nucleotide exchange factors (GEF) and GTPase activating protein (GAP), respectively. When the Ras protein changes GTP into GDP, it is deactivated (turned off). The protein does not transmit signals to the cell's nucleus when it is bound to GDP. As a result, mitosis cell division is always on. It is an important physiological role of Ras.

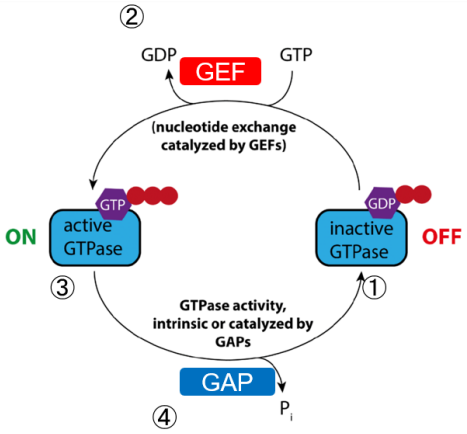

Fig. 1 Ras GTPase cycle GEF (Guanine nucleotide Exchange Factor). and GAP (GTPase Activating Protein) are control factors of Ras.

2. Aim of this Study

The aim of this study is to control Ras function photo reversibly using the engineered GAP modified with photoswitching molecular devices. Recently, we were able to photocontrol GTPase activity by introducing azobenzene derivatives to the motor domain's functional sites and H-Ras Hypervariable region[1]. In this study, three kinds of thiol-reactive azobenzene derivatives, N-(4-phenylazophenyl) maleimide (PAM), Amino-azobenzene-Maleimide (AABM) and Sulfonate-azobenzene-Maleimide (SABM) which exhibit different properties electrostatically, were employed as a photo switching molecular device. Furthermore, in order to incorporate the azobenzene derivatives into the functional site of GAP, the GAP mutant which have a reactive cysteine residue at the functional region of GAP.

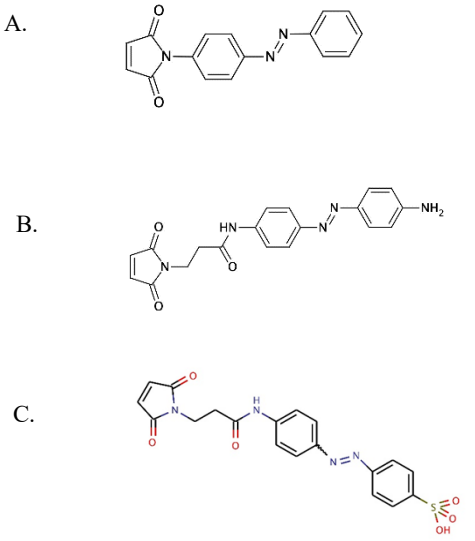

Fig. 2 Azobenzene derivatives as a Photochromic nanodevices. (A) Neutral charge photochromic nano device (PAM). (B) Positively charge photochromic nano device (AABM). (C) negatively charge photochromic nano device (SABM).

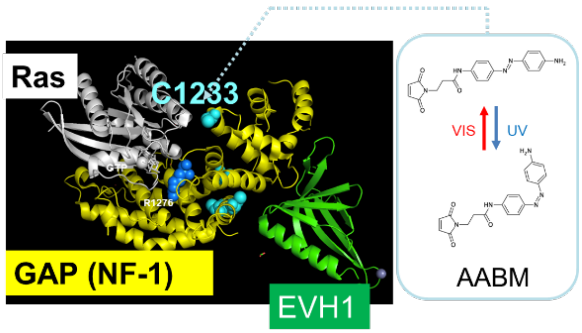

Fig. 3 Photoreversible-control of Ras using GAP mutant directly modified with photochromic molecules**.**

3. Materials and Methods

Preparation of Nf1:

Nf1 was prepared as previously described (Rufiat *et al*., 2021). Briefly, cDNA NF1 was amplified by polymerase chain reaction and ligated into the pET21a vector. NF1 expression plasmids were transformed into Escherichia coli Rosetta2 (DE3) pLysS (Invitrogen, CA, USA). NF1 were purified with Co2+-NTA columns. 55. Purified NF1 in the buffer of 150 mM NaCl, 1 mM MgCl2, 30 mM Tris-HCl (pH 7.5). It was stored in the deep freezer (-80 ℃).

GTPase assay:

The modified NF1- PAM was irradiated using VIS & UV light at 25C, 5 min before initiating the GTPase assay. Added GTP & incubated 30 min at 25 degrees (in the dark) & added 100ul 10% TCA to stop the reaction. The mixture was vortex well and stored on ice and did centrifuge for 5 min at 4C at 15,000 rpm. Then, 50ul sample was taken & added 100ul Biomol in the microplate reader & incubated for exactly 30min at 25 degrees and measured abs at 630nm.

4. Results and Discussion

The azobenzene derivatives, PAM, AABM and SABM were stoichiometrically incorporated into the GAP (C1233). The incorporated azobenzne derivatives exhibited Cis-Trans isomerization. AABM-GAP and PAM-GAP exhibited photoregulation of Ras GTPase slightly but not significantly. SABM-GAP showed almost no effect on the photoregulation of Ras GTPase. The other positions of GAP located at the interface between GAP and Ras should be modified with the azobenzene derivatives to perform efficient photo regulation.

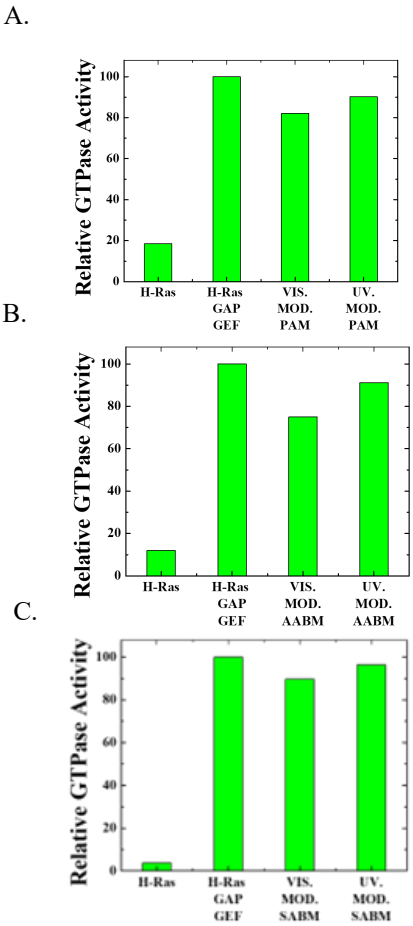

Fig. 3. Photocontrol of GTPase activity of H-Ras by GAP modified with azobenzne derivatives. The GAP modified with azobenzne derivatives was irradiated using VIS & UV light at 25C, 5 min before initiating the GTPase assay., measured abs at 630nm. (A) GAP Modified with PAM. (B) GAP Modified with AABM. And (B) GAP Modified with SABM.

5. Conclusion

GTPase activity of Ras using GAP modified with photochromic nanodevices was controlled by UV and visible light light irradiation reversibly.

6. Future Plan

Design of the various kind of GAP mutants such as L1300C, S1355C, and P1359C is performe. Photo control of Ras function with the designed GAP mutants modified with azobenzene derivatives is examined.

7. Reference

1. Nahar R, Noor A. A. M. D, Alrazi I. M. D, Maruta S. Photocontrol of GTPase Cycle and Multimerization of the Small G-Protein H-Ras using Photochromic Azobenzene Derivatives. Biosci Biotech Res Asia (2021) 18, 661-672.