Studies on the functional role of HVR Domain in the small G-protein and its application to Artificial control of Ras

Small G-Protein Research Group
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1. Background and Aim of Study

The small G protein Ras is a central regulator of cellular signal transduction processes, functioning as a molecular switch. Ras have a specific functional domain HVR. HVR determine the important physiological role of Ras. In this study, functional characterization of HVR was studied, and based on the properties, photocontrol of the Ras function was performed by incorporating designed photochromic nanodevices into HVR. Modification of cysteine residues as lipidation sites in HVR with caged compounds induced multimer of Ras. Electron microscopic observation and averaging image analysis of the multimer showed circular ring shape which is consistent with the structure estimated from X-ray scattering. In order to photocontrol Ras , the two different polarity photochromic sulfhydryl-reactive azobenzene derivatives, 4-phenylazophenyl maleimide (PAM) and 4-chloroacetoamido-4'-sulfo-azobenzene (CASAB) were incorporated into HVR. GTPase of the H-Ras modified with CASAB was photocontrolled more effectively than PAM-H-Ras. Interestingly PAM modification induced H-Ras multimerization, but not CASAB. In this study, it has been demonstrated that incorporating photochromic molecules as a regulatory nanodevice into the functional HVR domain enable to control Ras function photoreversibly.

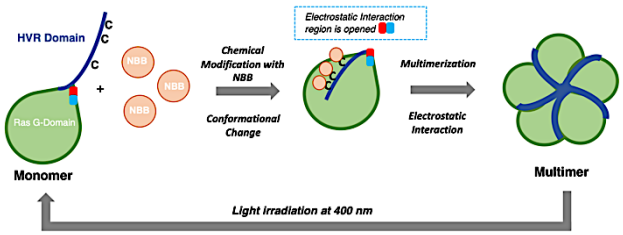

Fig. 1 Proposed possible mechanism for multimerization of H-Ras with NBB.

2. Materials and Methods

Preparation of C118S H-Ras The cDNA of human H-Ras WT (1-189) plasmids offered by Dr. Sako (RIKEN), was amplified by PCR and incorporated into the pET42c vector. Ras expression plasmids were transformed into Escherichia coli Rosetta2 (pLysE). The Ras was purified according to the established methods (1) with a Co2+-NTA column.

Structural analysis of NBB-Ras multimer by SAXS and TEM [SAXS] SAXS measurements were performed at beam line BL8S3 of the Aichi Synchrotron Radiation Center. [TEM] NBB-H-Ras or H-Ras were stained with 2% (w/v) uranyl acetate on a carbon- coated grid for 1 min at 23 ◦C, and the solution remained on the grid was removed and dried. The resultant grids were transferred into a transmission electron microscopy (JEM1010; JEOL) operated at 80 kV.

Photocontrol of Ras GTPase assay The effect of isomerization of azobenzene derivatives bound to the HVR of Ras on the GTPase activity in the presence of GAP and GEF has been examined . The transition between cis and trans isomers of PAM or CASAB on C118S was achieved by UV and visible light irradiation.

2. Results and Discussion

2-1. Structural analysis of NBB-Ras multimer by X-ray small angle solution scattering and dummy-atom structural modeling

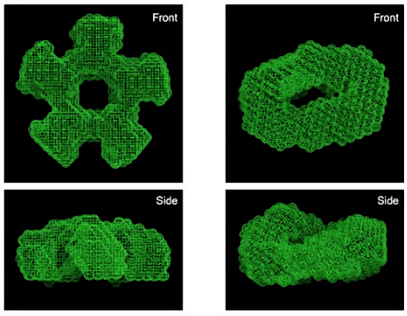

Fig. 2 Possible structures estimated from small angle X-ray scattering.

To study the global conformation of the multimer of H-Ras induced by NBB modification at HVR, we utilized a small-angle synchrotron X-ray scattering using synchrotron radiation. H-Ras (2, 4, 6, and 8 mg/ml) modified with NBB in 120 mM NaCl, 30 mM Tris-HCl, pH 7.5, and 1 mM $MgCl_2$ were measured at 25 °C.

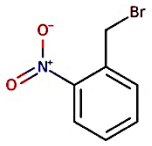

Fig. 3 Structure of NBB

2-2. Electron Microscopic observation of the Ras multimer.

Using negative staining and transmission electron microscopy, we observed the configurations of the NBB-H-Ras multimer and monomer. H-Ras modified with NBB showed apparently round-shaped particles with a diameter of approximately 20 nm, which is consistent with the size of the multimer estimated from P(r) function X-ray scattering. Only indistinguishable small particles, which may correspond to the monomer of H-Ras, were observed.

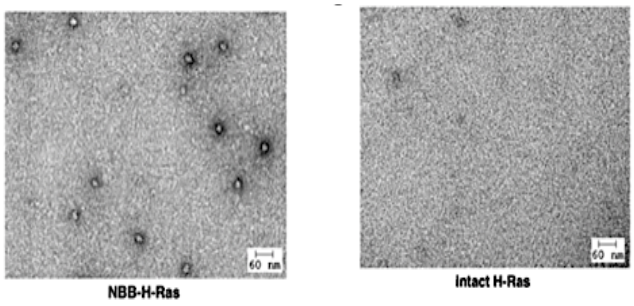

Fig. 4 Electron microscopic observation of Ras multimer.

2-3. Photocontrol of GTPase cycle of H-Ras modified with PAM and CASAB

The isomerization of PAM-Ras and CASAB-H-Ras modified with PAM and CASAB was performed at 0°C in modification buffer using UV light irradiation for induction of the cis state for 3 min and using Vis light irradiation of the trans state for 10 min and then repeated. The GTPase activity of PAM-Ras was measured at 25 °C.

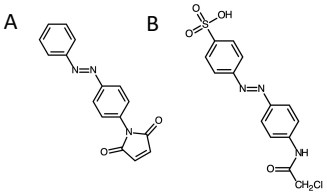

Fig. 4 Structure of PAM(A) and CASAB(B)

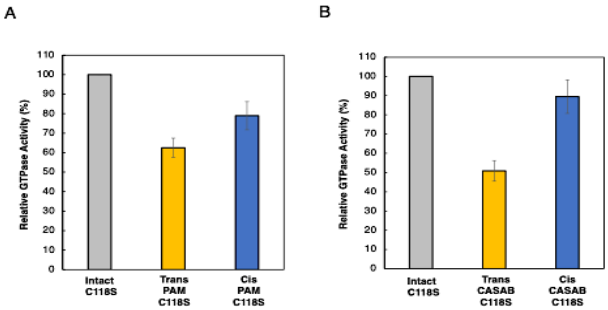

Fig. 5 Photocontrol of GTPase activity modified with PAM and CASAB

2-3. Reversible Photoregulation of C118S H-Ras and multimer formation.

CASAB-C118S changed its GTPase activity more significantly than PAM-C118S, accompanied by photoisomerization. Trans-CASAB-C118S reduced the GTPase activity to 62% of intact-C118S. Cis-CASAB-C118S exhibited almost the same GTPase activity (95%) as intact C118S. Azobenzene changes its molecular size and polarity drastically accompanied by cis-trans isomerization, as a result PAM modified H-Ras showed multimer formation and less efficient GTPase regulation. However, CASAB-H-Ras did not formed multimer but higher GTPase regulation.

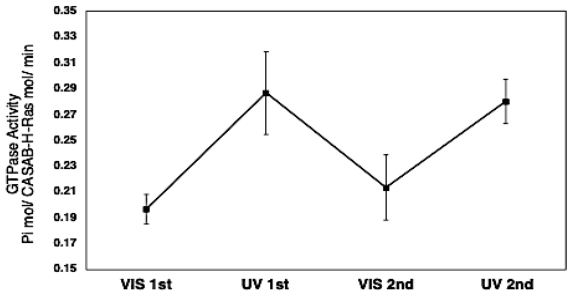

Fig. 6 Reproducible reversibility of the alteration of GTPase activity of the C118S modified with CASAB.

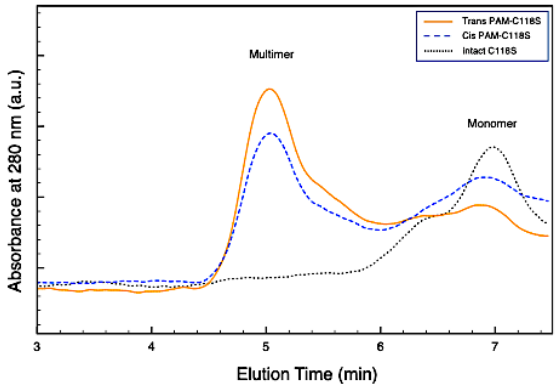

Fig. 7 Monitoring the H-Ras multimer induced by PAM.

Conclusion

We have successfully demonstrated that incorporating thiol-reactive photochromic azobenzene derivatives into the lipidation site in HVR, which is one of the physiologically functional sites of H-Ras, enables the reversible control of GTPase activity and multimer formation by UV and visible light irradiation.

References

(1) Ishikawa, K., Tohyama, K., Mitsuhashi, S., and Maruta, S. (2014) J. Biochem. 155, 257-263