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Title of Doctoral Dissertation

Design and Synthesis of a Multi-tag Exchangeable Nosyl-type Diazirine in Photoaffinity Labeling
（マルチタグ交換可能な新規ジアジリン光アフィニティープロブの創製）

Photoaffinity labeling has been widely used as an alternative tool to identify the molecular interactions between bioactive molecules and its target protein. Although this method has many advantages, identification of the target protein is still a challenging task. Hence, this study of ours focuses on the development of nosyl-type diazirines, which can be used as an alternative photoprobe in photoaffinity labeling. Phenyl diazirine with Ns scaffold was designed with two functionalities which are 1) form a cross-linked and 2) install different tags.

Thus, in order to prove the feasibility of our approach, Ns diazirine bearing amino ethanol, biotin and methotrexate was synthesized via 9 steps. The key step of this synthetic strategy is the oxidative chlorination using 2,4-dichloro-5,5-dimethylhydantoin (DCDMH). Besides that, a few fluorophores such as BODIPY and coumarin were also modified by introducing thiol functionality. Aminoethanol bearing Ns diazirines was used as model compound to prove its superiority in terms of photoreactive and kinetic photolysis in comparison with the normal trifluoromethyl diazirine. These compounds were irradiated under black light (30W) in methanol and the half life were calculated. Based on the results obtained, Ns diazirine photolyzed more rapidly than the normal phenyl diazirine.

Next, the evaluation on tag-exchange reaction was performed using coumarin-thiol as tag in search of the best condition for further application in physiological condition. Finally, the examination on the utilities of the Ns diazirines were carried out using Ns diazirine bearing biotin and methotrexate (MTX). Both compounds were irradiated under 365 nm light (250 W) at 0oC after being incubated in avidin (for biotin Ns diazirine) and dihydrofolate reductase (DHFR) (for MTX Ns diazirine). The photolabeled BSA was subjected to western blot using HRP peroxidase-conjugated avidin and the cross-linking efficiency was calculated as approximately maximum of 20%, determined by comparing with the calibration curve of biotinylated BSA. Subsequently, the photolabeled BSA and DHFR were subjected to SNAr with BODIPY thiol. Based on the emission intensity of the bodipythioether BSA band, the formation of photolabeled BSA was obtained with estimated value of approximately 78% yield after 24h. As for MTX Ns diazirine, detection of DHFR cross-linked photoprobe fluorescence band was detected at 21 KDa.

In conclusion, the bifunctional Ns diazirine based photoaffinity labeling probe was successfully synthesized and proved to exihibit higher photoreactivity in comparison with the normal phenyldiazirine. Additionally, this probe also proves the feasibility of the tag-exchangeable reaction via formation of Meisenheimer complex by installing various modified thiol tags.