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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)

In early stage of drug discovery, identifying the molecular interactions between the bioactive compound and its target protein is one of the crucial steps. For these purposes, some strategies have been implemented such as genetics approach, 3D structure determination involving solution state NMR and X-ray crystallography. Although these approaches can provide a detailed structural information of target biomolecule, an abundant purified molecule is always required. As an alternative, chemical method using affinity-based chemical probe such as photoaffinity labeling (PAL) has also been used for direct identification and visualization of target biomolecules in complex systems without having to undergo purification process. Phenyl diazirines are one of the important photolabile moieties used in photoaffinity labeling due to their superior photo labeling characteristic. Despite that, identification of the cross-linked target molecules often involves complexity of reaction mixtures and resulting in small quantity of the photo-labeled molecules. Therefore, the photolabeled molecule requires its enrichment by using fluorous tags, biotin-avidin system and clickable group. Besides that, multi-functional crosslinkers having high sensitive functions such as scissile, fluorescence and stable isotope also showed an excellent performance with multiple steps. Ideally, analysis of photolabeled molecules should be more direct and reliable.

With regard to this, our study focuses on the design and synthesis of novel nosyl bearing diazirines with scissile function which can simultaneously exchange various tags by S_NAr with an effort to provide more direct and reliable analysis. Intrigued by works done by Fukuyama et al. on the nosyl protection group, the photoaffinity probe was designed with Ns scaffold with two functional properties which 1) form a cross-linked and 2) install different tags. Also, the installation of the trifluoromethyl diazirine on the meta position enhances photolysis and facilitates the S_NAr reaction.

In order to evaluate the feasibility of our approach, model compounds Ns diazirine and modified thiol tags were synthesized. Key intermediate of benzyl mercaptan diazirine was synthesized through 8 steps before coupled with aminoethanol, biotin and methotrexate via oxidative chlorination using 2,4-dichloro-5,5-

dimethylhydantoin (DCDMH). 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. The results revealed that Ns diazirine was found out to be photolyzed more rapidly in comparison to normal diazirine as the half life ($t_{1/2}$) of these compounds were calculated as 2.5 and 4.5 min respectively. This strengthen the fact that Ns diazirine exhibited suitable characteristics for the photoaffinity reagent.

Next, the evaluation on the properties of exchangeability were performed by using thiol coumarin as tag with the presence of different basses such as triethylamine, $NaHCO_3$ and $CsCO_3$ in several organic solvents and H_2O. Based on these results, the S_NAr reaction proceeded effectively with the presence of $CsCO_3$ in aqueous media which means it is further applicable under physiological conditions. Further examination on the utilities of Ns diazirines using BSA as the model protein. Thus, biotinylated diazirine was incubated with BSA, photoirradiated and further subjected to 10% SDS-page gel. Then, it was detected with chemiluminescence method using horseradish peroxidase (HRP)-conjugated avidin after blotting on PVDF membrane. Based on the emission intensity, the cross-linking efficiency was calculated as approximately maximum of 20% determined by comparing it with the calibration curve of biotinylated BSA. Subsequently, The photolabeled BSA was subjected to S_NAr with bodipy thiol. Based on the emission intensity of the bodipy-thioether BSA band, the formation of photolabeled BSA was obtained with estimated value of approximately 78% yield after 24h.

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.