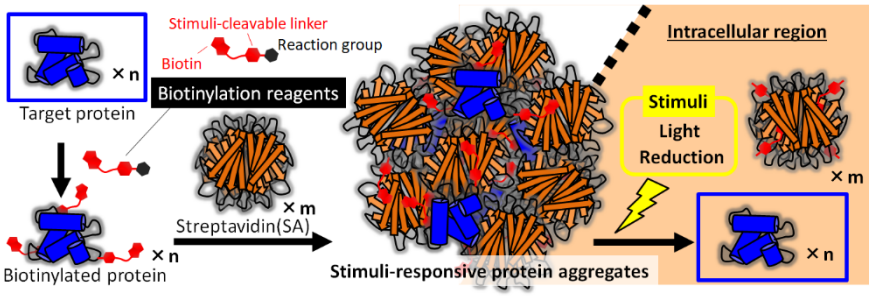
細胞内タンパク質送達を指向した刺激溶解性タンパク質凝集体の開発

(東大院工[1]・東大先端研[2]) ○山本一穂[1]・山口 哲志[2]・岡本 晃充[1,2]
Development of stimuli-degradable protein aggregates for intracellular protein delivery ([1]*Graduate School of Engineering, The University of Tokyo*, [2]*Research Center for Advanced Science and Technology*) ○Kazuho Yamamoto,[1] Satoshi Yamaguchi,[2] Akimitsu Okamoto[1,2]

Protein delivery into the target cells contributes to a variety of medical technologies. Reversibly-soluble protein aggregates have recently attracted attention as carriers for stably and selectively delivering high-concentration proteins[1)]. Our group has also developed photolytic protein aggregates consisting of biotinylated target proteins and streptavidin (SA) by using a photo-cleavable biotinylating reagent, and reported light-induced release of the target proteins in extracellular regions[2)]. In this study, we aimed to use the stimuli-responsive protein aggregates for intracellular protein release. Saporin (Sap), a ribosome-inactivating protein, was used as a model protein. The biotinylation reagents were a photodegradation type and a new reductive degradation type. As a result, we were able to prepare small aggregates of less than 100 nm, and their dissolution upon stimulation was confirmed. Furthermore, light-induced death of the cells transfected with photolytic Sap aggregates was observed to be achieved by non-toxic light (360 nm, 3 J/cm^2), confirming the photo-release of Sap in the cells.
Keywords : Drug Delivery System; Protein Aggregates; Protein bodies; Stimuli-degradable; Cancer Therapy

タンパク質の標的細胞内への送達技術は、幅広い医療技術に貢献する。近年、高濃度タンパク質を安定かつ選択的に送達するためのキャリアとして可逆的に溶解できるタンパク質凝集体が注目されている[1)]。我々も、光分解性ビオチン化試薬を用いて、ビオチン化標的タンパク質とストレプトアビジン(SA)から成る光溶解性ナノ凝集体を開発し、細胞外での光依存的な標的タンパク質の放出を報告してきた[2)]。本研究では、この刺激応答性タンパク質凝集体を用いて、細胞内でのタンパク質放出を試みた。モデルタンパク質として、リボソーム不活性化タンパク質であるサポリン（Sap）を用いた。ここで、ビオチン化試薬には光分解型と新たに還元分解型も用いた。条件検討の結果、100 nm 以下の微小な凝集体が調製でき、刺激による溶解も確認された。また、光溶解性 Sap 凝集体を導入した細胞において、無毒な光（360 nm, 3 J/cm^2）による光依存的な細胞死誘導も観察され、細胞内での Sap の光放出が確認された。



1) Xu, *et al.*, *JACS.* **2012**, *134*, 8774; 2) Ishiwatari, *et al.*, *Adv. Healthcare Mater*. **2016**, *5*, 1002

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