pGH Tag for Protein Purification

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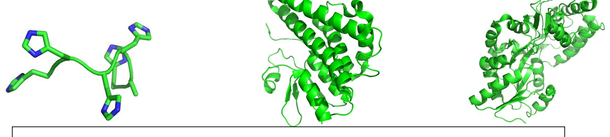
Protein Purification

Investigation of proteins, a biopolymer composed by amino acids as a building block, became increasingly important because of its relevance to various disease such as cancer, for example. In order to obtain fundamental insight into the function, development of expression and isolation of proteins has become essential. Tag strategy, appending peptide tag onto the target protein by genetic engineering, enabled quick and easy purification through reversible binding interaction between the tagged protein and functionalized beads (e.g. MBP, GST, and Hexa-His tag). However, currently available peptide tags requires introduction of long polypeptide chain. Herein, I present a new dipeptide tag, "pGlu-His" for protein purification.

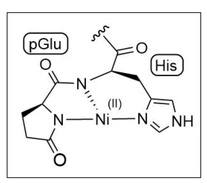
His-tag **6AAs** | GST-tag **~209AAs** | MBP-tag **~370AAs**



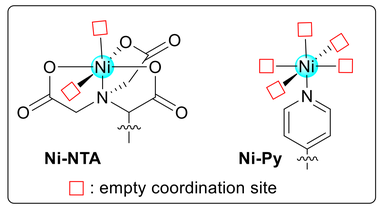
This Work
Pyroglutamate-histidine (pGH) tag **2AAs**

Pyroglutamate-histidine tag

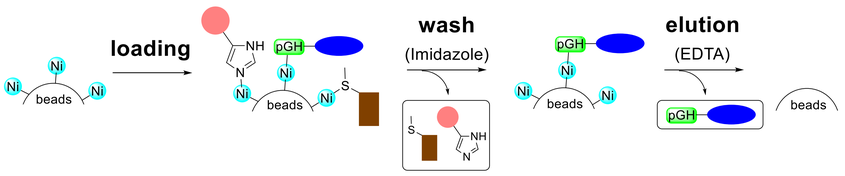
The Ball lab previously reported strikingly high reactivity of "pGlu-His" peptide for copper-mediated cross coupling reaction.[1] We hypothesized that the high reactivity of pGH tag is due to strong binding of the tag to divalent transition-metal ion (e.g. Cu or Ni). Although Ni-nitrilotriacetic acid (NTA) beads (with 2 coordination site on Ni) are commonly used for purification of His_6-tagged protein, the binding motif of pGH necessitates at least 3 binding coordination site on the metal center. Therefore, I have examined three different types of ligands for nickel to facilitate binding of pGH-tagged protein to the beads.



Putative binding structure of pGH and Ni(II)

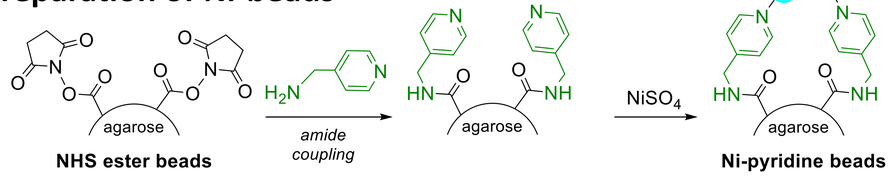


Proposed purification process

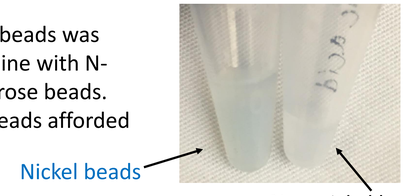


Methods and Results

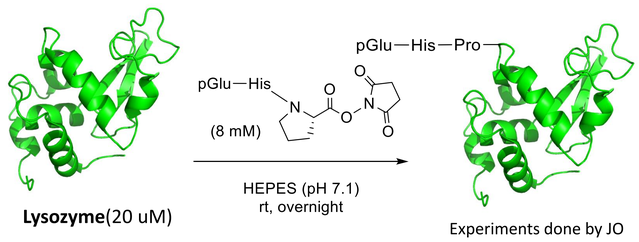
Preparation of Ni-beads



Preparation of the coordinatively unsaturated nickel beads was accomplished by reacting amine-functionalized pyridine with N-hydroxylsuccinimide (NHS)-activated acid on the agarose beads. Addition of nickel sulfate into the pyridine-agarose beads afforded the desired nickel beads.



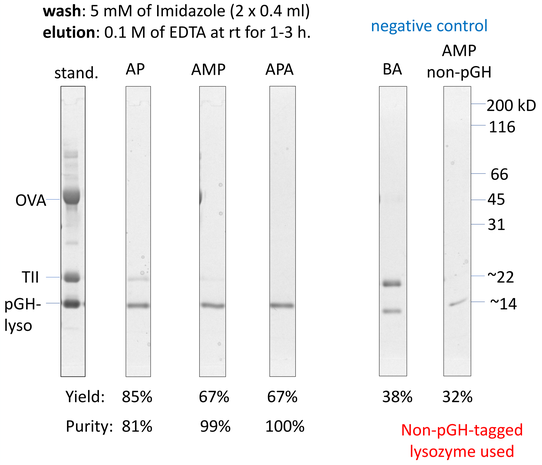
Preparation of pGH-lysozyme

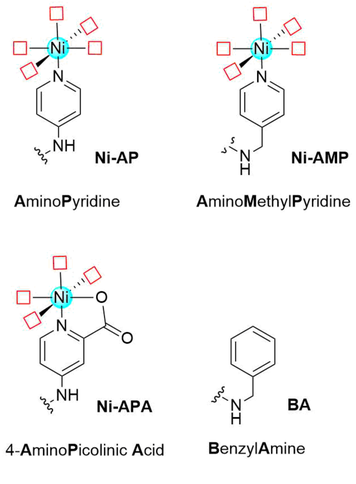


Pyroglutamate-histidine-tagged protein was produced by reaction of NHS-activated tripeptide (pGHP) and lysozyme through non-specific NH_2-selective chemistry. Mass-spectroscopy confirmed roughly single modification of the protein.

Testing three kinds of ligands

loading: 20 uM of lysozyme, TII, OVA in NMM buffer (50m M, pH 11, total 0.4 mL) at rt for 1-3 h.
wash: 5 mM of Imidazole (2 x 0.4 ml)
elution: 0.1 M of EDTA at rt for 1-3 h.

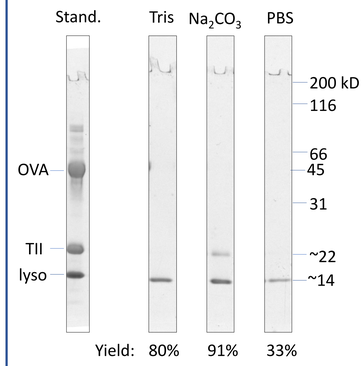




As an initial trial, we have examined three types of pyridine based ligand (AP, AMP, and APA). The purification experiment was conducted as shown on the left using a mixture of three proteins: ovalbumin (OVA), trypsin inhibitor I (TII), and pGH-tagged lysozyme (pGH-lyso). The eluted solution was analyzed by SDS-PAGE, demonstrating isolation of the tagged protein in good yield (67~85%).

However, negative control experiments with non-pyridine based beads (BA) or non-pGH-tagged lysozyme also shows presence of the lysozyme in the eluted solution (in 32~38% yield), presumably due to nonspecific binding of the protein onto agarose beads.

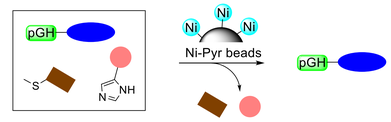
Buffer Screening



It is essential to suppress nonspecific binding of lysozyme, and thus I performed buffer screening to find an optimal condition.

As a result, phosphate buffered saline (PBS) proved most effective to minimize the nonspecific binding.

Conclusion&Future Research

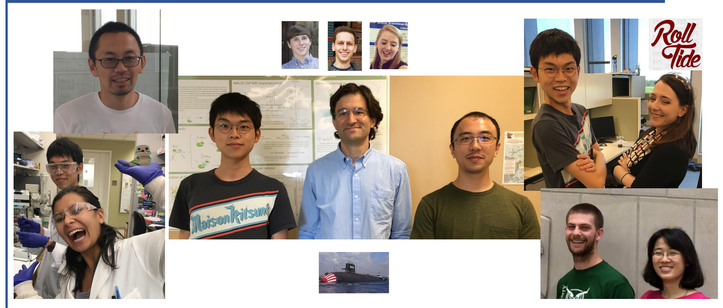


In conclusion, purification of pGH-tagged protein was demonstrated. Although the non-tagged protein displayed nonspecific binding to the beads, I discovered that use of PBS buffer successfully solve the issue.
Future study will focus on further optimization of the purification process. Specifically, purification of other pGH-tagged proteins proteins such as TII would an useful experiment. Moreover, we are working on the expression pGH-tagged protein by genetic engineering as well.

Reference

1) Ohata, J.; Minus, M. B.; Abernathy, M. E.; Ball, Z. T. *J. Am. Chem. Soc.* **2016**, *138*, 7472.

Acknowledgement



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