ON/OFF repetitive control of protease activity using peptidomimetic ligands through biotin-streptavidin affinity

Koushi Hidaka, Keiko Hojo, Yuko Tsuda

Kobe Gakuin University, Japan

<https://doi.org/10.17952/35EPS.2018.173>

Introduction

Peptidomimetic protease inhibitors targeting the enzyme active site are useful as research tools and drugs. However, such small compounds are difficult to remove from the proteases once they bound, requiring large dilution or protein denaturation procedures. Our research group developed “removable inhibitors” that was designed with a direct conjugation of biotin and a strippable property, through extremely strong affinity to streptavidin (SA) (1) even after the binding to the target protease. This inhibitor stripping action was caused by affinity competition between target protease and SA, named as ISAAC. To extend the utility of removable inhibitors, we studied the ON/OFF repetitive control of enzymatic activity derived from affinity-purified proteases.

Results and Discussion

Previously, we reported a removable inhibitor, bPI-11 which was synthesized from a HIV protease inhibitor, a derivative of KNI-10006 containing an amino group by coupling with biotin using mixed anhydride (2). Enzymatic activity of HIV-1 protease was inhibited by bPI-11, which was sufficiently recovered by addition of SA, and then inhibited again by extra addition of the inhibitor. The result showed ON/OFF/ON/OFF repetitive control of the enzymatic activity of HIV protease (Figure 1).

Based on this result, we performed the activity detection of the affinity purified protease. Recombinant HIV-1 protease was mixed with human serum. The target protease was purified using magnetic beads conjugated with the inhibitor with a long aminocaproyl spacer. The bound protease was eluted with a solution containing bPI-11 and the eluate was examined for the activity detection using FRET substrates by ISAAC. As we expected, the substrate cleavage was confirmed by addition of SA and suppressed by following addition of clinical protease inhibitors such as darunavir, lopinavir and atazanavir (Figure 2). Similar experiments using a mutated protease, A17m5, which is lopinavir-resistant, were performed. We detected the lower enzymatic activity and slightly different inhibition by the clinical drugs from that of the wild-type protease. Some biotinylated derivatives of pepstatin A, were also utilized to detect cathepsin D activity purified from HCT116 cells and the activity was inhibited by addition of pepstatin A.

Figure 1: Repetitive control of protease activity using bPI-11.

Figure 2: Activity detection of wild-type HIV protease mixed in with human serum.

In conclusion, we confirmed that a directly biotinylated protease inhibitor, bPI-11, repetitively controlled HIV protease activity. We also succeeded in detecting the enzymatic activity after the affinity purification from human serum and inhibited by clinical drugs. These results suggest that ON/OFF repetitive control of protease activity using removable inhibitors could be applied for testing susceptibility and drug resistance before the treatment with protease inhibitor drugs.

Acknowledgements

This research was supported by a grant from JSPS KAKENHI, 18K05344.

References

1. Howarth, M., et al., Nat. Methods, 3, 267-273 (2006).
2. Beck-Sickinger, A. G. and Diederichsen, U. (Eds.) Proceedings of the 34th European Peptide Symposium, The European Peptide Society, Leipzig, 2017, p. 178.