Live-Cell Protein Lysine Acylation Using Boronate-Assisted Hydroxamic Acid Catalyst

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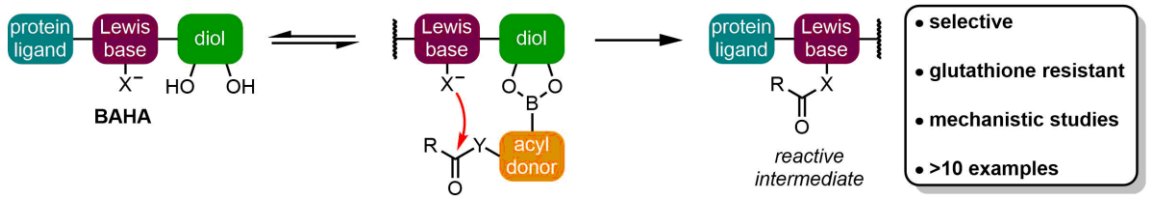
Eukaryotic cell processes including signal transduction, regulation of enzyme activity, and transcriptional regulation are tightly regulated via protein post-translational modifications (PTMs). Thus, chemical methods to install PTMs within living cells should prove useful as tools for manipulating these phenomena. Our research group has reported regioselective protein lysine acylation using the DSH catalyst system.[1] However, competition between the DSH catalyst and glutathione (GSH) leads to a lower concentration of the reactive intermediate. This necessitated millimolar concentrations of thioester acyl donor. However, exposure of cells to thioester reagents at millimolar concentrations leads to considerable off-target acylation.

To overcome this problem, we devised the boronate-assisted hydroxamic acid (BAHA) catalyst system (Figure). In this system, the catalyst's diol module reversibly binds to a boronic acid-containing acyl donor. The subsequent intramolecular acyl transfer to a Lewis base moiety thus benefits from a local molarity effect. A more rapid pathway to the reactive intermediate increases the concentration of the reactive intermediate, thus enabling a high yielding reaction even at micromolar reagent concentrations. Critically, this process is not significantly inhibited by GSH.

To showcase this methodology, we demonstrated the installation of naturally occurring and abiotic lysine acylation within HEK293T cells. As off-target protein acylation is very low when compared to precedent methods, the BAHA system constitutes a minimally invasive tool for lysine PTM installation within living cells.



1) Amamoto, Y. *et al. J. Am. Chem. Soc.* **2017**, *139*, 7568.