DOCTORAL THESIS

Design and Synthesis of Cell Permeable Activatable Fluorescence Probes for Detecting Cyclic ADP-ribose Synthase Activity via Base-Exchange Reactions

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ABSTRACT

Cyclic ADR-Ribose Synthases (cADPRSs) are one of the NAD-consuming enzymes. It hydrolyzes and cyclizes NAD^+ into ADPR and cADPR, which are both Ca^{2+} channel regulators. Since the activities of cADPRSs involves in multiple biological process, monitoring the activities of these enzymes can provide useful information for studying pathology of the diseases that are closely associated with these enzymes. In this thesis, we have designed and synthesized a series of small molecule probes which can undergo base exchange reaction with the nicotinamide group of NAD^+ in the presence of CD38 or activated SARM1. A large red shift of emission wavelength occurred after the base exchange reaction, which provides a powerful tool for detecting activities of this class of enzymes.

In the first project, these probes were applied to the detection of the activities of SARM1. Among the 23 probes prepared, **PC6** and **PC11** showed excellent sensitivity and selectivity *in vitro*. They are cell-permeant, yet the resulting exchange products are impermeant, allowing imaging of activated SARM1 in live cells. **PC6** has provided the first evidence that SARM1 activation precedes axon degeneration by several hours in live DRG neurons. Moreover, it was also applied in the library screening for SARM1 inhibitor. Dehydronitrosonisodipine (dHNN) was found to has the inhibition ability to SARM1 activation, which is also the first compound ever reported that can inhibit SARM1 activation. **PC11** has better fluorescent properties than **PC6**. With larger absorption and emission wavelength, **PC11** provided the first approach for imaging SARM1 activation *in vivo*.

In the second project, we focused on the study of the catalytic mechanism of CD38. Based on the preliminary results of the theoretical studies, we proposed that CD38-catalyzed cyclization and hydrolysis of NAD^+ may involve an epoxide intermediate. In this mechanistic study, we have employed our newly developed probe **PC6**, CD38 mutant and different model compounds. The results of this study strongly supported the hypothesis of an epoxide intermediate in CD38-catalyzed reactions.