Abstract 1783

Probing protein arginine methyltransferase (PRMT) activity in mammalian cell lysates

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The activities of the family of nine mammalian protein arginine methyltransferases (PRMTs 1–9) are often overexpressed in cancer but it has been challenging to determine the *in vivo* substrates of each of these enzymes, especially with their potential overlapping substrate specificity. Remarkably, it has not been possible to date to readily assay the activity of the individual PRMTs in cell extracts. However, the development of inhibitors for PRMTs with specificity for the individual enzymes has now made possible new approaches. In this study we demonstrate the capture of specific types of PRMT activities in mammalian cell lysates utilizing a P81 phosphocellulose assay and a radioactive gel assay based on newly developed PRMT inhibitors. To parse out potentially unique activities of PRMTs, we have characterized recombinant human PRMT1, PRMT5, and PRMT7 activities *in vitro*. PRMT1 represents the major type I PRMT activity responsible for forming asymmetric dimethylarginine residues, PRMT5 represents the major type II activity forming symmetric dimethylarginine residues, and PRMT7 is the only type III activity limited to forming monomethylarginine residues. We have identified potential regulation of these enzymes by temperature, pH, and ionic strength. PRMT1 has optimal activity at a temperature range of 20 °C–37 °C, a pH of 7.4, and an ionic strength of 0 M. PRMT5 has optimal activity at a temperature of 37 °C, pH at a wide range of 6.6–8.5, and an ionic strength of 0 M. PRMT7 has an optimal activity at a temperature of 15 °C, a pH of 8.5, and an ionic strength of 0 M. Using these conditions with the type I specific inhibitor MS023 at 2 μM, the PRMT5 inhibitor EPZ015666 at 20 μM, and the PRMT7 inhibitor SGC8158 at 0.2 μM, we have measured PRMT activities in mammalian cell lysates. Altogether, our results suggest that PRMT1 is the main enzyme responsible for arginine methylation which supports previous results using knockout cells. However, the activity of PRMT5 in mammalian lysates is quite low in comparison, while PRMT7 activity is difficult to detect. The ability to demonstrate that individual PRMT activities in mammalian cell lysates may be useful to discern the relevance of PRMTs in normal cells and in cancer cells.

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Abstract 1856

Investigating the role of the N-terminal cysteine-rich domain of violaxanthin de-epoxidase like –2 (VDL-2) in redox sensing in diatoms

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Carotenoid pigments in photosynthetic organisms serve both light harvesting and energy dissipating roles. One key enzyme involved in regulating the response of photosynthetic organisms to high light intensity is violaxanthin de-epoxidase, which converts the light harvesting carotenoids violaxanthin to zeaxanthin under high light conditions. Compared to land plants, diatoms contain a wide range of additional carotenoids, such as fucoxanthin and diadinoxanthin, which require an additional suite of enzymes to biosynthesize and regulate. The diatom enzyme violaxanthin de-epoxidase like-2 (VDL-2) has previously been reported to be involved in the biosynthesis of fucoxanthin, the key light harvesting carotenoid in diatoms. VDL-2 is closely related to land plant violaxanthin de-epoxidase enzymes, which share an N-terminal cysteine-rich domain involved in regulation. In this study, we investigate the activity of full-length recombinant VDL-2 from the diatom *Thalassiosira pseudonana* to identify the role of disulfide bonds in the N-terminal domain in regulating enzymatic activity. We successfully isolated full-length VDL-2 enzyme through Ni-NTA and S-200 size exclusion column chromatography. Surprisingly, the purified enzyme has a distinct yellow color from an unknown chromophore absorbing at approximately 406 nm. We utilized 5,5′-dithio-bis-(2-nitrobenzoic acid) to identify the oxidation state of the cysteine residues in the N-terminal domain, and the impact of changes in solution redox poise on the number of free thiols. In the future, we plan to correlate redox state and structure of the N-terminal cysteine-rich domain to the rate of carotenoid turnover of the enzyme.

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