Mechanistic Studies on the Aminopeptidase from *Vibrio proteolyticus.*

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Leucine aminopeptidases have been implicated in tumor growth and metastasis, HIV infectivity, immunomodulation and bacterial infectivity. Inhibitors of these enzymes have been shown to possess immunomodulatory and anticancer activity. A detailed mechanism of action has been proposed, based on EPR spectroscopic studies of Co(II)-substituted forms of the native dizinc aminopeptidase from *V. proteolyticus* and on x-ray crystallographic studies. These studies relied on the ability of various inhibitors to generate species that appear to mimic the important intermediates in the catalytic cycle. However, to date there are no studies of complexes with *bona fide* substrates of the enzyme and the proposed mechanism remains somewhat speculative.

In our continuing work on this enzyme, we have explored the nature of the catalytic cycle using both peptide substrates and inhibitors. Samples with substrates were generated using rapid freeze quench and analyzed using EPR spectroscopy. Further studies have probed the determinants of substrate specificity of the enzyme through mutational analysis of residues adjacent to the active site, by kinetic and spectroscopic methods.