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Structural and functional studies of Assimilatory Nitrite Reductase

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1 Introduction

Nitrogen is an essential element required for the synthesis of physiological substrates, including nucleic acids and proteins. Higher plants acquire nitrogen, primarily as NO_3^-, from the soil. The NO_3^- is preserved in the vacuoles before being sequentially reduced to NH_4^+ by two enzymes: assimilatory nitrate reductase and assimilatory nitrite reductase (aNiR). The produced NH_4^+ is used by glutamine synthetase and glutamate synthetase in amino acid synthesis. These processes are known as nitrogen assimilation. In comparison with dissimilatory nitrite reductase (dNiR) for the alternative inorganic nitrogen metabolic pathway, the molecular mechanism of the reduction for aNiR has hardly been shown yet.

2 Structure-function relationship of assimilatory nitrite reductases from the leaf and root of tobacco based on the high resolution structure [1, 2]

Tobacco expresses four isomers of assimilatory nitrite reductase (aNiR), leaf-type (Nii1 and Nii3) and root-type (Nii2 and Nii4). The high resolution crystal structures of Nii3 and Nii4, determined at 1.25 and 2.3 Å resolutions respectively, revealed that both proteins had very similar structures. Although these structures are almost identical to spinach aNiR that were previously obtained at 2.8 Å resolution by Knaff & Allen's group, the Nii3 structure provided detailed geometries for the [4Fe-4S] cluster and the siroheme prosthetic groups. We have generated two types of Nii3 variants: one set focuses on residue Met175 (Nii3-M175G, Nii3-M175E and Nii3-M175K), a residue that is located on the substrate entrance pathway; the second set targets residue Gln448 (Nii3-Q448K), a residue near the prosthetic groups. Comparison of the structures and kinetics of the Nii3 wild-type (Nii3-WT) and the Met175 variants showed that the hydrophobic side-chain of Met175 facilitated enzyme efficiency (k_{cat}/K_m). The Nii4-WT has Lys449 at the equivalent position of Gln448 in Nii3-WT. The enzyme activity assay revealed that the turnover number (k_{cat}) and Michaelis constant (K_m) of Nii4-WT were lower than those of Nii3-WT. By combining detailed crystal structures with enzyme kinetics, we have proposed that Nii3 is the low-affinity and Nii4 is the high-affinity aNiR.

3 The reductive reaction mechanism of tobacco nitrite reductase derived from a combination of crystal structures and ultraviolet-visible microspectroscopy [3]

A reaction mechanism for Nii3, an aNiR from tobacco, is proposed based on high resolution X-ray structures and UV-Vis microspectroscopy of Nii3-ligand complexes. Analysis of UV-Vis spectral changes in Nii3 crystals with increasing X-ray exposure showed prosthetic group reductions. In Nii3-NO_2^- structures, X-ray irradiation enhanced the progress of the reduction reaction, and cleavage of the N-O bond was observed when X-ray doses were increased. Crystal structures of Nii3 with other bound ligands, such as Nii3-NO and Nii3-NH_2OH, were also determined. Further, by combining information from these Nii3 ligand-bound structures, including that of Nii3-NO_2^-, with UV-Vis data obtained at different X-ray doses, a reaction mechanism for aNiR was constructed.

4 X-ray crystal structure of a mutant assimilatory nitrite reductase that shows sulfite reductase-like activity [4]

aNiR reduces nitrite to ammonium, whereas assimilatory sulfite reductase reduces sulfite to hydrogen sulfide. Although aNiR can also reduce sulfite, its activity is much lower when nitrite is reduced as the substrate. To increase the sulfite reduction activity of aNiR, we performed a N226K mutation of Nii3, a representative aNiR. The resulting Nii3-N226K variant could bind non-native targets, SO_3^{2-} and HCO_3^-, in addition to its native target, NO_2^-. We have determined the high-resolution structure of Nii3-N226K in its apo-state and in complex with SO_3^{2-}, NO_2^- and HCO_3^-. A comparison of all ligand bound structures for Nii3-N226K revealed that structural changes in active site depend on the size of the substrate.

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