Structure of hydrazine synthase, an electron transfer complex

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Research aims

Wastewater treatment using anaerobic ammonium oxidation (anammox) bacteria is an innovative nitrogen treatment technology, and its application to actual wastewater has begun. In the anammox reaction, nitrite is reduced by ammonia as the electron donor, after passing through hydrazine (N_2H_4) as a reaction intermediate, and nitrogen molecules are produced ($NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$). However, the reaction mechanism is not fully understood. In particular, the N_2H_4 synthesis reaction is found only in anammox bacteria, and its details were completely unknown until relatively recently. In 2011, there was a report on hydrazine synthase (HZS, a heterotrimeric heme protein) as an enzyme for the synthesis of N_2H_4 from NH_3 and nitrogen monoxide (NO)[1)]. In the present study, the reaction product N_2H_4 was not detected directly, and the reaction rate was very low. However, we purified another heme protein, NaxLS (a heterodimer consisting of NaxL and NaxS subunits) [2)], and determined its three-dimensional structure. The structure revealed that the heme iron of NaxLS is coordinated by His/Cys, and indicated that NaxLS works as a specific electron transfer protein within the anammox reaction. We attempted a hydrazine synthesis reaction using HZS and NaxLS, and detected the production of N_2H_4 from NH_3 and NO as substrates. It was proposed that the electron transfer reaction from NaxLS to HZS is an indispensable step in hydrazine synthesis. From these results, NaxLS was suspected to function as a physiological electron transfer protein for HZS. In the present study, we attempted to reveal the structure of the HZS-NaxLS electron transfer complex, using X-ray crystal structure analysis.

Methods

HZS and NaxLS were purified from an enrichment culture of the anammox bacterium strain KSU-1, via column chromatography. Using a mixture of both proteins, crystallization conditions were determined via the vapor diffusion method. Approximately 200 conditions were investigated, using a commercial screening kit. The obtained crystals were analyzed using SDS-PAGE, and complex crystals were frozen and stored in liquid nitrogen. Diffraction data were collected at the SPring-8 BL44XU and BL41XU, and were integrated and scaled using HKL2000 and XDS. The structure of the HZS-NaxLS protein complex was solved using a combination of Fe-SAD phasing and the molecular replacement method.

Results

After performing crystallization procedures with a mixture of the HZS and NaxLS proteins, single crystals of the complex of both proteins were obtained, using pentaerythritol ethoxylate and polyvinylpyrrolidone as the precipitants (Figure 1). X-ray diffraction data for these crystals were then obtained at a resolution of 2.8 Å. Using the molecular replacement method and the crystal structure of HZS[3], we were able to determine the crystal structure of the HZS-NaxLS protein complex (Figure 2). HZS formed a dimer of the heterotrimer αβγ, and one NaxLS molecule was bound

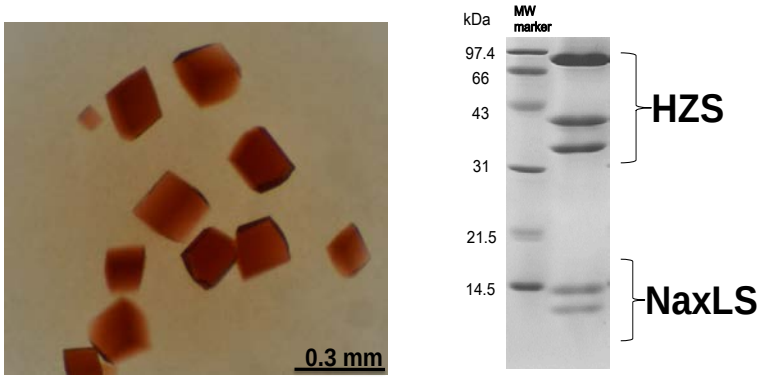

Figure 1. Crystals of the HZS-NaxLS protein complex (left) and SDS-PAGE results of the crystals (right).

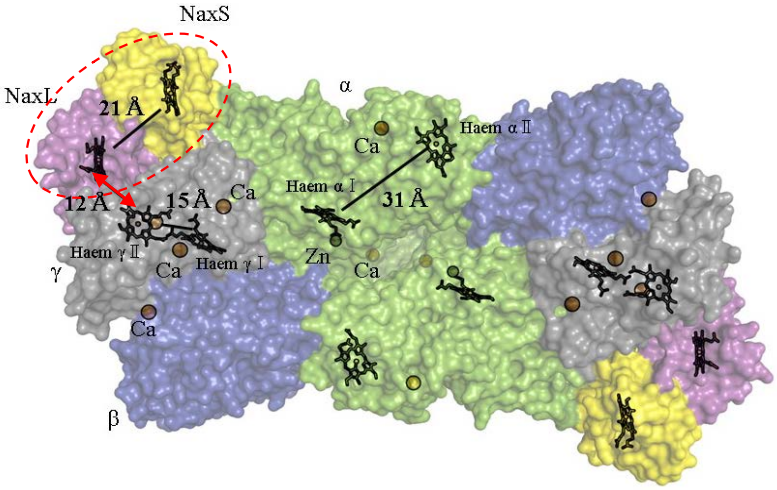

Figure 2. Crystal structure of the HZS-NaxLS protein complex. The red dashed line indicates the NaxLS molecule.

to the heterotrimer (αβγ) of HZS. In addition, we determined that a heme coordinated by the His/Cys of the NaxL subunit of NaxLS, and a 6 coordinated heme of the γ subunit of HZS, were located at a distance of 12 Å. We determined that an intermolecular electron transfer reaction is possible through these hemes.

Conclusion

N_2H_4 synthesis from NH_3 and NO requires the transfer of 3 electrons. From the revealed structure of the hydrazine synthase complex consisting of HZS and NaxLS, and from biochemical experimental results, we hypothesized that a two-electron-transfer reaction from NaxLS to HZS is an essential step in the hydrazine synthesis reaction. Our next goal will be to elucidate the effects of formation of this protein complex on the mechanisms of hydrazine synthesis reaction, based on the known structure of this complex.

References

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