Roles of charged residues in pH-dependent redox properties of cytochrome c3 from Desulfovibrio vulgaris Miyazaki F

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Complicated pH-properties of the tetraheme cytochrome c3 (cyt c3) from Desulfovibrio vulgaris Miyazaki F (DvMF) were examined by the pH titrations of 1H-15N HSQC spectra in the ferric and ferrous states. The redox-linked pKa shift for the propionate group at C13 of heme 1 was observed as the changes of the NH signals around it. This pKa shift is consistent with the redox-linked conformational alteration responsible for the cooperative reduction between hemes 1 and 2. On the other hand, large chemical shift changes caused by the protonation/deprotonation of Glu41 and/or Asp42, and His67 were redox-independent. Nevertheless, these charged residues affect the redox properties of the four hemes. Furthermore, one of interesting charged residues, Glu41, was studied by site-directed mutagenesis. E41K mutation increased the microscopic redox potentials of heme 1 by 46 and 34 mV, and heme 2 by 35 and 30 mV at the first and last reduction steps, respectively. Although global folding in the crystal structure of E41K cyt c3 is similar to that of wild type, local change was observed in 1H NMR spectrum. Glu41 is important to keep the stable conformation in the region between hemes 1 and 2, controlling the redox properties of DvMF cyt c3. In contrast, the kinetic parameters for electron transfer from DvMF [NiFe] hydrogenase were not influenced by E41K mutation. This suggests that the region between hemes 1 and 2 is not involved in the interaction with [NiFe] hydrogenase, and it supports the idea that heme 4 is the exclusive entrance gate to accept the electron in the initial reduction stage.

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