D-Amino acid related electron transfer system in the hyperthermophile

*Pyrobaculum islandicum*.

Chihiro Suzuki, Ko-hei Saito, Asami Uehara, Minoru Tanigawa, Yoko Nagata
Dept. of Mater. and Appl. Chem., Coll. of Sci. and Technol., Nihon Univ., Tokyo,
Japan, chibita0@kg8.so-net.ne.jp

(Purpose of study)

*Pyrobaculum islandicum* is a strictly anaerobic archaeon that grows optimally at 100 °C. The organism grows both organotrophically on complex organic compounds such as peptone, and lithotrophically using H₂ as an electron donor and sulfur as an electron acceptor (Huber et al., Arch Microbiol (1987) 95-101). We previously reported on the D-amino acid contents and D-amino acid dehydrogenase (DAD) activity of *P. islandicum* (Nagata et al., Biochem. Biophys. Acta 1435 (1999) 160-166). In the present study, we purified cytochromes from *P. islandicum* cells, since the respiration chain of the organism is not known. And then, we performed a reconstitution experiment using purified DAD and cytochromes in order to confirm the electron transport route between a D-amino acid and the final electron acceptor.

(Methods)

The cells were disrupted by a French press, and the homogenate was centrifuged to remove unbroken cells. The membrane fraction obtained by ultracentrifugation (140,000 g × 60 min) of the cell-free extract was solubilized with 0.1% Tween 20 to obtain the DAD fraction. DAD was purified by chromatographies with DEAE-Toyopearl, hydroxyapatite and Sephacryl S-100. The insoluble fraction after the Tween 20-treatment, was further solubilized with 5% Triton X-100. The b-type cytochromes were separated from the solubilized fraction by ammonium sulfate fractionations and purified by ion-exchange chromatography using CM-Toyopearl followed by hydroxyapatite in chromatography. Electron transport to the b-type cytochrome was assessed spectrophotometrically.

(Results)

A peak of reduced form of a b-type cytochrome appeared at around 560 nm, upon addition of D-valine to the mixture containing the b-type cytochrome and DAD, followed by incubation at 50°C for 5 min.

(Conclusions)

The transport route of electrons originated from D-valine, and the cytochrome involved in the route were elucidated in *P. islandicum*.