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Stability and function of a thermophilic cytochrome c'

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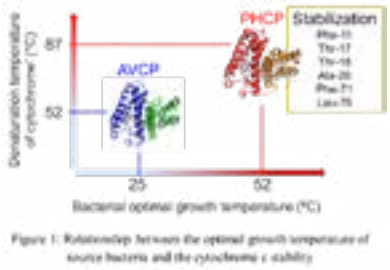
Cytochromes c' are classified as heme proteins found in restricted Gram-negative bacteria. They usually form a homo dimeric structure, and the single subunit typically consists of four helix bundle. Biochemical analysis showed that they can bind diatomic gasses such as NO or CO, but not O_2. Recently we purified cytochrome c' from thermophilic *Hydrogenophilus thermoluteolus*, and named it PHCP. *H. thermoluteolus* grows optimally at 52°C, indicating that PHCP is more stable than homologous proteins from mesophiles. In this study, we compared stability and function of PHCP with its mesophilic homologue, *Allochromatium vinosum* cytochrome c' (AVCP) having 55 % amino acid sequence identity. In order to check the stability, we measured the circular dichroism spectra with increasing temperature. The denaturation temperature of PHCP was 87°C, which was higher than that of AVCP (52°C). The X-ray structure comparison between PHCP and AVCP revealed that the stability difference was due to the heme-related interactions and subunit-subunit interactions, which was also proofed by mutagenesis study. These results indicated that PHCP advantageously retains the native structure at high temperature. The PHCP X-ray structure further revealed a ligand binding channel and a penta-coordinated heme, as observed in the AVCP protein, indicating PHCP could bind diatomic gasses at high temperature. Thus, we measured the gas binding affinity of PHCP and AVCP using absorption spectra. The association constant (Ka) of PHCP with CO was 3 times lower than that of AVCP at 25°C, and PHCP could maintain normal spectral changes up to 60°C. In AVCP, such spectral changes with CO could not to be detected at 60°C, because of denaturation of AVCP. In conclusion, PHCP has a structure fulfilling the requirement for both gas-binding function and thermal stability. This stable cytochrome c' will become a model for protein engineering field.



Recent Publications

1. Fujii S, Masanari M, Inoue H, Yamanaka M, Wakai S, Nishihara H, Sambongi Y (2013) High thermal stability and unique trimer formation of cytochrome c' from thermophilic *Hydrogenophilus thermoluteolus*. Biosci Biotechnol Biochem 77:1677-1681.
2. Fujii S, Masanari M, Yamanaka M, Wakai S, Sambongi Y (2014) High stability of apo-cytochrome c' from thermophilic Hydrogenophilus thermoluteolus. Biosci Biotechnol Biochem 78:1191-1194.
3. Kato Y, Fujii S, Kuribayashi TA, Masanari M, Sambongi Y (2015) Thermal stability of cytochrome c' from mesophilic Shewanella amazonensis. Biosci Biotechnol Biochem. 80: 2365-2370.
4. Fujii S, Oki H, Kawahara K, Yamane D, Yamanaka M, Maruno T, Kobayashi Y, Masanari M, Wakai S, Nishihara H, Ohkubo T, Sambongi Y (2017) Structural and functional insights into thermally stable cytochrome c' from a thermophile. Protein Sci. 26: 737-748.

Biography

Sotaro Fujii is working on the stability, structure, and function of proteins that are important for microbial energy metabolism. A characteristic aspect of his research activity is comparison of the homologous proteins isolated from microorganisms living in extreme environments in which humans cannot live and those isolated from 'normal' environments.

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