NISR RESEARCH GRANT
2004 Research Grant

Function of Thermostable Redox Proteins as Thermo-electric Energy Converters

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Research aims

Purpose of this study is elucidation of molecular property of biological energy metabolism in an aspect of application science and technology. We will deal with a thermally stable redox protein, cytochrome *c*, and apply it to use as a thermo-electric energy converter. We have established basic technology in handling of various cytochromes *c*[1]. Here we try to understand functional mechanism of cytochrome *c*, and gain insight into a novel strategy in industrial energy production.

Methods

This study consists of three sub-projects.

(1) Structure, stability, and electron transfer reaction of cytochromes *c*: Five cytochromes *c* from five Gram-negative bacteria, (I) *Shewanella violacea* (growing at 8°C), (II) *Pseudomonas aeruginosa* (37°C), (III) *Hydrogenophilus thermoluteolus* (52°C), (IV) *Hydrogenobacter thermophilus* (72°C), and (V) *Aquifex aeolicus* (85°C), were used in this study. These cytochromes *c* were tested for thermal and chemical denaturation using circular dichroic (CD) spectroscopy and for redox function derived from electron transfer reaction using cyclic voltammetry (CV).

(2) Cytochrome c expression: We tested how the cytochromes *c* (I to V above) were able to be expressed in *Escherichia coli* efficiently used as a host organism. We examined gene structure to be expressed, and selected suitable *E. coli* host strains.

(3) Temperature dependent redox potential: We measured temperature dependency of redox potential of the cytochromes *c* by CV. Furthermore, we searched for engineered cytochromes *c* that had larger difference in redox poteintial during temperature change.

Results

(1) Structure, stability, and electron transfer reaction of cytochromes *c*: We found that the order of stability of cytochromes *c* from bacteria (II) to (V) was the same as that of the growth temperature of original bacteria[2]. The stability of protein from (I) was the same as that of (II). This is because that the cytochrome *c* from (I) has a unique SS bond, which contributes to the enhanced stability. Furthermore, the protein from (V) was extremely stable; it was not denatured at 130°C, or with 7 M denaturant, guanidine hydrochloride at pH 5.0.

We also measured redox potential at 25°C for the proteins from (II) and (IV). We were able to unify the free energy change during redox reaction and that during protein denaturation[3].

(2) Cytochrome *c* expression: The cytochrome *c* from (II) was expressed in *E. coli*, and the yield reached up to 30 mg product per 1 liter cell culture. However, the other cytochromes *c* were not expressed when the intact genes were introduced into *E. coli*. We found that these cytochromes *c* were expressed in *E. coli* when the genes were fused with the periplasmic targeting signal pepetide from the (II) protein[2].

Furthermore, the expression of most cytochromes *c* was dependent on Dsb proteins which were responsible for heme attachment in *E. coli* cells. Exceptionally, however, the protein from (V) was not dependent on the Dsb proteins, and the expression level was higher without the Dsb proteins than with them[4].

(3) Temperature dependent redox potential: We found that redox poteintials of all the proteins tested here were higher at lower temperature than at higher temperature[5]. These results indicate that the proteins can generate electricity with changing temperature. Furthermore, we found that a variant of (III) protein carrying single mutation showed elevated stability and larger temperature dependency in redox potential.

Conclusion

The progress of this study is that we gain insight into (i) relationship between stability and redox poteintial in cytochromes *c*, (ii) maximization of cytochrome *c* expression, (iii) possibility in generating electric power using cytochromes *c* with temperature change. These findings may be a base in developing thermo-electric energy converters using electron-transferring proteins, cytochromes *c* which are thermally stable.

References

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