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CYTOCHROME C-550 FROM A THERMOPHILIC BACTERIUM PS3

Koyu Hon-nami, Tairo Oshima, Hiroshi Kihara* and Yasuo Kagawa*

Mitsubishi-Kasei Institute of Life Sciences, Minamiooya, Machida, Tokyo 194
and
*Jichi Medical School, Minamikawachi-machi, Tochigi 329-04 (Japan)

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SUMMARY: A c-type cytochrome, cytochrome c-550, was highly purified from a moderately thermophilic bacterium PS3. The absorption peaks were at 550, 521, and 416 nm in the reduced form, and at 408 nm in the oxidized form. The isoelectric point was at pH 5.6, and the molecular weight was about 10,000. The heat stability of the cytochrome was intermediate between those of horse cytochrome c and cytochrome c-552 from an extreme thermophile, Thermus thermophilus HB8.

INTRODUCTION

It has been established that cell components including biopolymers and cell organellae of thermophiles are more heat-stable than those of mesophiles (1). The stable membrane components of thermophiles have been very useful in reconstitution studies on energy-transforming biomembranes (2). During such studies, cytochrome c's of thermophiles have been purified and characterized and it has been shown that cytochrome c-552 from an extreme thermophile, Thermus thermophilus HB8, is more stable than horse cytochrome c (3,4).

The present paper describes the purification and some properties of cytochrome c-550 from the moderate thermophile PS3, and a comparison of its stability with those of cytochrome c's from horse and T. thermophilus HB8.

MATERIALS AND METHODS

Bacterium and its membrane — The thermophilic bacterium PS3 was cultured and its washed membranes were obtained by the methods used previously for preparation of DCCD-sensitive ATPase (5,6).

Cytochrome c's — Highly purified cytochrome c-552 from T. thermophilus HB8 was prepared as previously reported (3). Horse cytochrome c (Type VI)

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was purchased from Sigma Co. (USA). Concentrations of cytochrome c's were determined spectrophotometrically on the basis of the following extinction coefficients: cytochrome c-552, ε_{522} nm (reduced) = $21.1\times10^{3}M^{-1}cm^{-1}$(3); horse cytochrome c, ε_{550} nm (reduced) = $27.7\times10^{3}M^{-1}cm^{-1}$(7). The molar extinction coefficient at the α-peak of cytochrome c-550 was calculated on the basis of that for the pyridine ferrohemochrome of heme c, $29.1\times10^{3}M^{-1}cm^{-1}$(8).

RESULTS AND DISCUSSION

Purification of cytochrome c-550 — Membranes of the thermophile PS3 were solubilized with 2% Triton X-100 and 0.2 M Na_2SO_4, and the extracts obtained by centrifugation were diluted with distilled water and applied to a DEAE-cellulose column as previously reported (6). Fractions not adsorbed on the column were pooled, concentrated, and then dialyzed against 5 mM Tris-HCl buffer, pH 8.5. The dialyzate was applied to a DEAE-cellulose column equilibrated with the same buffer and eluted with the same buffer containing 0.1 M NaCl. The eluate was dialyzed agianst 50 mM Tris-HCl buffer, pH 8.5, and then applied to a DEAE-cellulose column (2×38 cm, Whatman DE-32). The chromatogram was developed with a linear gradient formed using 500 ml of the same buffer and 500 ml of 0.2 M KCl in the same buffer. Red-colored fractions were pooled, concentrated, and then chromatographed on a Sephadex G-75 column (1.5×90 cm) with 10 mM phosphate buffer, pH 7.0. Fractions of eluate with ratios of A_{408nm} to A_{280nm} of more than 4.5 were subjected to isoelectric focusing using 1% carrier Ampholyte (LKB, Sweden) in the pH range of 4.0-6.0 for 70 h. The isoelectric point of cytochrome c-550 was determined to be pH 5.6 at 4°C. The peak fractions were collected and dialyzed against an appropriate buffer. The purified preparation was homogeneous as judged by 7.5% polyacrylamide gel electrophoresis at pH 8.3 (9) and its ratio of A_{408nm} to A_{280nm} of 5.7.

Properties of cytochrome c-550 — The absorption spectra of the oxidized and reduced forms of purified cytochrome c-550 are shown in FIG. 1. None of the absorption maxima were in the near-ultraviolet region. The molar extinction coefficients at 550 and 416 nm in the reduced form, at 408 nm in the oxidized form, and at 550 nm in the reduced-minus-oxidized difference

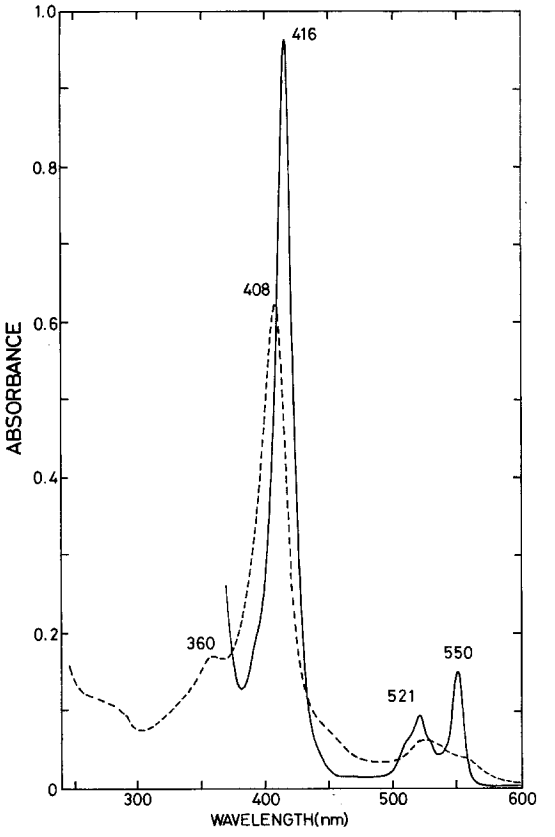

FIG. 1. Absorption spectra of purified PS3 cytochrome c-550. A solution of the cytochrome (5.5 μM) in 50 mM phosphate buffer, pH 7.0. was used ---, oxidized: ——, reduced with $Na_2S_2O_4$. Measurements were performed with a Cary 17 spectrophotometer.

spectrum were 27.5 and 174.2, 113.1, and 19.3 × 10^3 $M^{-1}cm^{-1}$, respectively. In the oxidized form the absorption spectrum exhibited a small peak at 690 nm with a molar extinction coefficient of 0.88 × 10^3 $M^{-1}cm^{-1}$. The heme of the cytochrome was not extracted with acidic methylethylketone (10), and its pyridine ferrohemochrome (8) showed absorption peaks at 415, 521 and 550 nm, indicating that this cytochrome is c-type. The low-temperature absorption spectrum of reduced cytochrome c-550 showed an asymmetric α-band with a maximum at 549 nm and a shoulder at about 547 nm. The circular dichroism spectrum in the oxidized form is shown in FIG. 2. Analytical ultracentrifugal studies indicated that the cytochrome preparation was

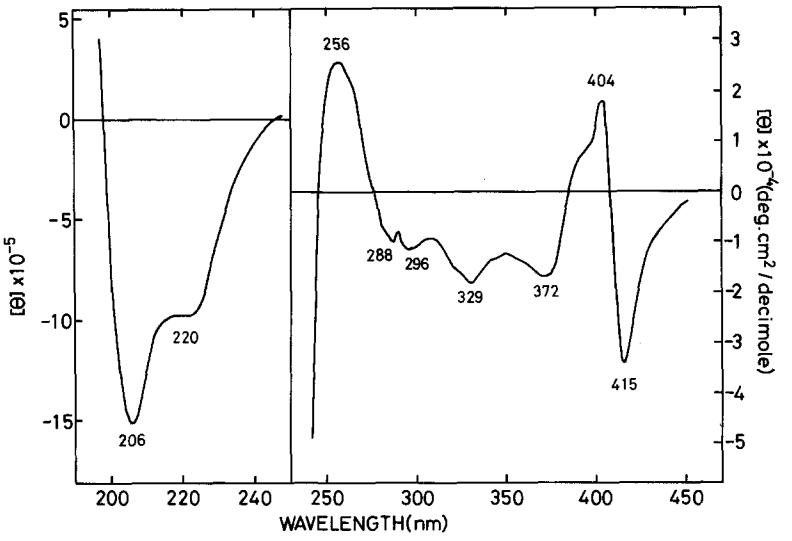

FIG. 2. Circular dichroism spectrum of oxidized cytochrome c-550. The cytochrome was dissolved in 50 mM phosphate buffer, pH 7.0, and the spectrum was recorded on a Jasco J-40A spectropolarimeter.

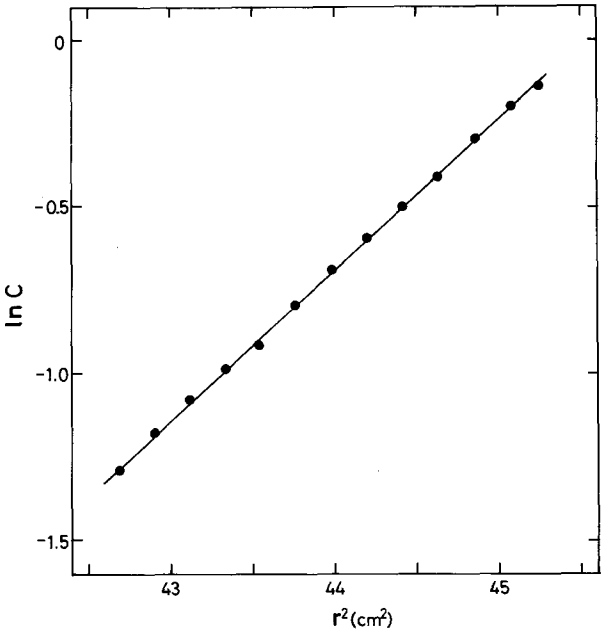

FIG. 3. Equilibrium sedimentation of PS3 cytochrome c-550. Centrifugation was carried out in a Hitachi 282 analytical ultracentrifuge equipped with a photoelectric scanner at a wavelength of 280 nm. The cytochrome (0.29 mg per ml in 50 mM phosphate buffer, pH 7.0) was equilibrated in a double-sector cell in a RA-72 TC rotor at a rotor speed of 27,063 rpm at 20°C. The natural logarithm of protein concentration (lnC) was plotted as a function of the square of the distance from the center of rotation (r^2).

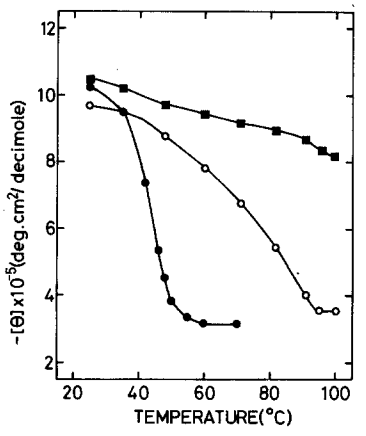

FIG. 4. Heat stability of cytochrome c's. Changes in the molar ellipticities at 222.5 nm with temperature were recorded with a Jasco J-40A spectropolarimeter. O , PS3 cytochrome c-550 (18.8 μM); ■ , cytochrome c-552 from Thermus thermophilus HB8 (21 μM); ● , horse cytochrome c (21 μM). Solutions of cytochromes in 50 mM phoshate buffer, pH 7.0, containing 2 M guanidine-HCl were used.

homogeneous. A sedimentation coefficient, $s_{20,w}$, of 1.56 S was obtained at a protein concentration of 0.38 mg per ml. Sedimentation equilibrium gave a molecular weight of 10,400 with an assumed partial specific volume of 0.73 (FIG. 3). Ferrocytochrome c-550 was slowly auto-oxidizable, and did not combined with CO at neutral pH. Cytochrome c-550 was oxidized by cytochrome oxidase of PS3, but not by that of beef heart (11).

Heat stability — Cytochrome c-550 was much more stable than horse cytochrome c, but less stable than Thermus cytochrome c-552; cytochrome c-550 and horse cytochrome c were completely denatured at 96 and 60°C, respectively, in the presence of 2 M guanidine-HCl, while Thermus cytochrome c-552 retained its ordered form to some extent even at 100°C (FIG. 4). The heat stabilities of these cytochrome c's seem to be positively correlated with the growth temperatures of the organisms from which cytochromes were extracted.

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