Purification and Some Physico-Chemical Properties of
Rhodopseudomonas spheroides Cytochrome 550

by Sigehiro MORITA*

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The high potential c-type cytochromes of photosynthetic bacteria (cytochrome c₂) were first isolated by Vernon¹. The cytochromes were commonly found in every species of photosynthetic bacteria, except for green bacteria²⁻⁴. Some of the cytochromes were highly purified and some of the physico-chemical properties were measured⁵⁻⁷⁻⁸. In Rhodopseudomonas spheroides, the high potential cytochrome was found and purified by Vernon and Kamen³, besides other hemproteins, i.e., b-type cytochrome⁹, cytochromoid c⁵⁻⁶ and a low potential c-type cytochrome¹⁰. In this note, further purification of R. spheroides cytochrome 550 (high potential c-type cytochrome) is reported and some of the physico-chemical properties measured with the crystalline preparation of the cytochrome are also described.

Experimental and Results

A strain of R. spheroides was grown under semi-anaerobic condition, under constant illumination, in medium consisting of peptone (0.2%), yeast extract (0.1%) and sodium lactate (0.2%) at pH 7.0, 32°. The cells, harvested after 48 hours' culturing and washed once with sodium chloride solution, were used as the starting material.

Extraction

The cytochrome was extracted by three different ways; 1) by disruption of the cells by sonic oscillation (with Tohoriko, Type 50-5, 10 KC, 100 W, for 30 min.), 2) by extraction of the acetone-treated cells with a phosphate buffer solution (M/5, pH 7.0), and 3) by extraction with warm trichloroacetic acid solution, using the procedure of Vernon and Kamen³. All of these three methods gave good yields of the extracts.

Purification

The purification procedures were essentially the same with those used in the previous paper⁶. The procedures were a combination of fractional precipitation with ammonium sulfate and separation on a XE 64 ion-exchange resin column. The procedures were repeated, if necessary, until the ratio A₅₅₀ mp/A₂₇₅ mp of the preparation became as high as 0.8. Further purification was done by crystallization from a concentrated solution of partly purified preparation by the addition of a small amount of ammonium sulfate (Fig. 1). A preparation of the cytochrome with a value of the ratio A₅₅₀ mp/A₂₇₅ mp as high as 1.1 was thus obtained.

* Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan.
Fig. 1 Crystals of *Rhodopseudomonas spheroides* cytochrome 550 (reduced form).

Fig. 2 Absorption spectrum of *Rhodopseudomonas spheroides* cytochrome 550 (reduced form). Measured with a Cary Spectrophotometer Type 14. The molar absorbancy is expressed on a basis of heme concentration.
Spectral characteristics

The absorption spectrum of reduced form of the cytochrome is illustrated in Fig. 2. The absorption maxima of the ferro-cytochrome lie at 550 μm, 521 μm, 416 μm, 317 μm and 275 μm. The molar extinction values are calculated on a basis of heme concentration as estimated from the alkaline pyridine hemochromogen spectrum, by method of Bartsch. The values were $27.5 \times 10^3$ liter / mole, cm at 550 mμ and $129 \times 10^3$ liter / mole, cm at 416 mμ, respectively.

Oxidation-reduction potential

The oxidation-reduction potential of the cytochrome was measured by the usual spectro-photometric method, using ferri-ferro cyanide mixtures as oxidation-reduction buffers, in M/10 phosphate buffer, pH 7.0, at 20°. The value for the normal potential at pH 7.0, 20° was found to be 0.346 Volt, assuming the $E'_0$ value of ferri-ferro cyanide system to be 0.409 Volt.

Molecular weight

The molecular weight of the cytochrome was calculated from the sedimentation and free-diffusion constant. The sedimentation constant was determined with Spinco Ultra-Centrifuge Model E, in a synthetic boundary cell. In a phosphate buffer of pH 7.0 and ionic strength 0.2, the value for the sedimentation constant was computed to be $S_{20,w}=2.11 \times 10^{-13}$, extrapolating to zero concentration of cytochrome. The free-diffusion constant in the same medium was $D_{20,w}=1.35 \times 10^{-6}$ cm²/sec. From these values, the molecular weight of the cytochrome was calculated to be $1.3 \times 10^4$, assuming the partial specific volume of the cytochrome to be 0.71 ml/g.

Isoelectric point

The electrophoretic measurement of the cytochrome was carried out with a Tiselius-type apparatus (Hitachi Co. Ltd., Model HT-B) at 20°, and in phosphate buffer (ionic strength, 0.2). The isoelectric point of the cytochrome, as determined by interpolation to zero-mobility, was found to be pH 7.9.

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References


摘要

森田茂広：Rhodopseudomonas spheroides チトクロム 550 の精製とその物理化学的性質

1) 紅色細菌 Rhodopseudomonas spheroides の 550 μm に吸収をもつチトクロムを純化分画とイオン交換樹脂 XE 64 のカラムクロマトグラフにより精製した。さらに結晶化により純化を進めた。
2) 結晶チトクロムについて測定した結果、550, 523, 418, 317および278 mμ波長に吸収の極大をもち、モル吸光係数は 550 mμ で 28×10³, 418 mμ で 130×10³ であった。酸化還元電位は 0.346 Volt (pH 7.0, 20°C)、分子量は 1.3×10⁴、等電点は pH 7.9 であった。（東京大学理学部生物化学教室）