Presence of Cytochrome \(d\) in Respiratory Particles of *Streptomyces griseus* and its Function in the Terminal Oxidation System

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The cytochrome \(d\) in *Streptomyces griseus* was studied by means of difference spectra measured under dithionite- or substrate-reduction. Results indicated that the pigment 625 of *Streptomyces griseus* is a \(d\)-type cytochrome. Its pyridine hemochrome showed the difference maximum at 613 nm. The difference maximum of the reduced cytochrome \(d\) was shifted to the red by the addition of carbon monoxide or nitric oxide. Cytochrome \(d\) apparently acts as one of the respiratory pigments under aerobic conditions.

The respiratory particle showed nitrite reductase activity at the acidic pH, and the substrate-reduced cytochromes \(b\) and \(d\) were oxidized in the presence of nitrite under anaerobic conditions.

Sato (1940), Birk et al. (1957), Heim et al. (1957), Inoue (1958), and Niederpruem and Hackett (1961) reported the presence of cytochrome components in *Streptomyces*. However, no investigators reported on a \(d\)-type cytochrome in this microorganism. In 1973, Inoue reported that *S. griseus* contained a pigment which showed the difference maximum at 625 nm in addition to cytochromes \(a\), \(b\) and \(c\). This pigment was designated as the pigment 625. It was suggested by Inoue (1973) that the pigment 625 in the respiratory particle acted as one of the respiratory pigments and that the pigment 625 as well as other cytochrome components was reduced by the respiratory substrate and oxidized by oxygen. It was not clear, however, whether the pigment 625 was a \(d\)-type cytochrome. If the pigment 625 is a \(d\)-type cytochrome, this pigment should participate in the nitrite reduction system under anaerobic conditions.

This paper describes the spectrophotometric study of the cytochromes in respiratory particles (67P) of *S. griseus*, and discusses a function of the pigment 625 in the respiratory chain under aerobic and anaerobic conditions.

**Materials and Methods**

*Streptomyces griseus* Waksman and Henrici was used. Culture conditions for the organism were the same as described previously (Inoue and Kubo, 1965).

**Preparation of the respiratory particles**: Mycelium (8 g) was suspended in 80 ml of 0.2 M phosphate buffer (pH 7.4) and was exposed to sonic oscillation (20 Kc) for 10 min. The homogenate was centrifuged at 10000 \(\times\)g for 20 min. Then the supernatant was centrifuged at 67000 \(\times\)g for 1 hr. The precipitated particles were suspended in
4 ml of 0.2 M phosphate buffer (pH 7.4). This particle fraction was designated as the 67P and was employed throughout this study.

Preparation of lipase-treated 67P (lip-67P) and NaCl-67P: The preparation of lipase solution and the lipase-treatment procedure were conducted according to the method of Revsin and Brodie (1969). In this procedure, the mycelium was suspended in 80 mM Tris-HCl buffer (pH 8.0) and was exposed to sonic oscillation as described above. The supernatant of the homogenate at 10000 ×g was divided into two parts. One part was suspended in 12 ml of lipase solution containing 10% NaCl and was shaken at 37 C for 2 hr. Then the suspension was centrifuged at 67000 ×g for 1 hr, and the precipitated particles were suspended in 4 ml of 0.2 M phosphate buffer (pH 7.4). This particle fraction was designated as the lip-67P. Another part was suspended in Tris-HCl buffer containing 10% NaCl solution and was given the same treatment but without lipase. The particle fraction thus obtained was designated as the NaCl-67P.

For spectrophotometric measurements under aerobic conditions, the reaction mixture (1 ml) was placed in a cuvette of 1×1×4 cm (light path, 1 cm). The top of the cuvette was covered with Parafilm®, and the cuvette was turned upside-down exactly five times by hand. The difference spectrum was measured by a Beckman DB-G spectrophotometer at suitable intervals (usually every 5 min). The standard-scanning speed was 50 nm/min. The compositions of the reaction mixtures are described in the legends of figures. For anaerobic measurements, a Thunberg type cuvette (light path, 1 cm) was used. The reaction mixture (1.5 ml) was placed in a cuvette, and the substrate in the side arm. The tube was then evacuated by a vacuum pump until the outer surface of the cuvette became wet with a condensed water (usually 5 to 6 min was required). During evacuation, each cuvette was often shaken by hand for expelling the dissolved air. The substrate was added to the main chamber, and the assay was initiated. In the reference cuvette, the reaction mixture without substrate was placed. Though the volume of the reaction mixture was slightly reduced by the evacuation, the difference in volumes between experiments was negligible. Measurement of the difference spectrum of the dithionite-reduced 67P was started 3 min after the addition of the reductant. All experiments were repeated at least three times.

The activity of the nitrite reducing system following NADH oxidation under anaerobic conditions was measured spectrophotometrically as the change of optical density at 340 nm due to NADH oxidation.

Protein content was determined by either a biuret method (Gornall et al., 1948) or a Lowry’s method (Lowry et al., 1951).

Lipase (Steapsin) was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio; NADH was from Boehringer, Mannheim, Germany. Carbon monoxide and nitric oxide were obtained from commercial sources and were passed through an alkaline-pyrogallol solution to remove contaminating oxygen.