Nitrite reductase activity of *Pseudomonas* cytochrome oxidase

As is well known, *Pseudomonas aeruginosa* can be cultivated under anaerobic as well as under aerobic conditions, if nitrate is present. Thus the bacterium is able to use nitrate as an electron acceptor under anaerobic conditions, as well as oxygen under aerobic conditions; this is called nitrate respiration. From the bacterium, P-cytochrome *c*-551, P-cytochrome-554, P-cytochrome oxidase and P-blue protein have been extracted and purified, and P-cytochrome *c*-551 and P-blue protein obtained crystalline[1-5]. Another cytochrome, P-cytochrome-(560), which has its α-absorption band at 560 mμ, has also been found in the bacterium[1,6]. These respiratory components have been shown to function in nitrate respiration as well as in oxygen respiration of the organism[6]. P-cytochrome oxidase, which is almost pure ultracentrifugally and 70 % pure electrophoretically, contains two haem moieties, haem a_2 and a *c*-type haem[7,8], and oxidizes reduced P-cytochrome *c*-551 and P-blue protein, but not reduced P-cytochrome-554 or mammalian cytochrome *c*[2,7]. Like animal cytochrome oxidase, the enzyme also oxidizes L-ascorbate and hydroquinone. The oxygen consumption in the above reactions is inhibited strongly by a small amount of nitrite, but not by nitrate. This suggests either that nitrite is an inhibitor of P-cytochrome oxidase or that an electron destined for oxygen in the absence of nitrite is captured by nitrite. When nitrite and the oxidase were present together, reduced P-cytochrome *c*-551 could also be oxidized rapidly under anaerobic conditons, as shown in Fig. 1, and a part of nitrite present in the reaction mixture disappeared. Neither nitrate nor hydroxylamine could replace nitrite. This indicates that reduced P-cytochrome *c*-551 donates an electron to nitrite in the presence of P-cytochrome oxidase. This reaction was about 80 % inhibited by 10^{-3} *M* cyanide, which had been found to give complete inhibition of the oxidase activity under aerobic conditions. Heat treatment (70° for 5 min) of P-cytochrome

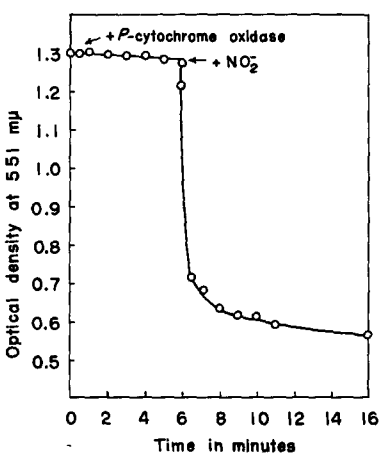

Fig. 1. Oxidation of reduced P-cytochrome *c*-551 by nitrite in the presence of P-cytochrome oxidase under anaerobic conditions. Reactions were carried out in a Thunberg tube modified for spectrophotometry. The tube has two side chambers. After the tube had been evacuated and filled with N_2, reduced P-cytochrome *c*-551 and P-cytochrome oxidase were mixed. The absorbancy at 551 mμ decreased very slightly. After 6 min, nitrite was added to the reaction mixture, bringing about a rapid oxidation of reduced P-cytochrome *c*-551. When P-cytochrome oxidase was absent, nitrite could not oxidize reduced P-cytochrome *c*-551, under the experimental conditions: pH 7.0, 28°.

oxidase diminished the velocity of the reaction to a tenth of the original. Hydroquinone could also act as electron donor for the reduction of nitrite. Nitrite was also reduced with yeast lactate dehydrogenase, lactate and P-cytochrome *c*-551 as an electron-donor system. If phenazine methosulphate was used instead of P-cytochrome *c*-551, nitrite reduction was much accelerated. However, in this case, cyanide did not inhibit. Pyocyanine, which is a pigment produced by the bacterium, could replace phenazine methosulphate, which may be a clue towards elucidating the physiological function of pyocyanine in the organism. None of the following haemoproteins could replace P-cytochrome oxidase in this reaction: P-cytochrome *c*-551, mammalian cytochrome *c*, cytochrome *c* modified by treatment with trichloroacetic acid[9], cytochrome *a*, a mixture of cytochrome *a* and cytochrome *c* which shows cytochrome oxidase activity[10], and catalase. With the hand spectroscope, P-cytochrome oxidase reduced with ascorbate could be seen to be oxidized immediately under anaerobic conditions when nitrite was added.

VERHOEVEN AND TAKEDA[11] observed that a reduced "cytochrome *c*"-type pigment in a sonicate of *Pseudomonas aeruginosa* was oxidized by addition of nitrite.

The pigment may be identical with P-cytochrome *c*-551. Our results with highly purified cytochrome components have extended these findings and have shown that P-cytochrome oxidase functions as nitrite reductase. This is in good accordance with the fact that P-cytochrome oxidase can be extracted in greater amounts from cells cultivated under anaerobic than from those grown under aerobic conditions.

The authors would like to express their thanks to Dr. T. HORIO (Graduate Department of Biochemistry, Brandeis University, Waltham, Mass., U.S.A.) for valuable discussions, and to Mr. K. MIKI for his technical assistance during this study.

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Received August 29th, 1960