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Purification and some properties of a c-type cytochrome from *Pseudomonas saccharophila*

We have shown that there are great differences between the enzymic properties of bacterial cytochrome *c*'s[1]. Thus, reduced Pseudomonas cytochrome *c*-551 (ref. 2) is rapidly oxidized by Pseudomonas cytochrome oxidase whereas reduced Rhodospirillum cytochrome c_2 (ref. 3) is very slowly oxidized by this enzyme. Therefore, it is interesting to see whether cytochrome *c*'s of different Pseudomonads differ especially with regard to their enzymic properties. In the present investigation, a *c*-type cytochrome was purified from *Pseudomonas saccharophila* and its properties were studied.

Acetone-dried cells of *P. saccharophila* (= *P. stutzeri*; strain IAM 1504 from the Institute of Applied Microbiology, Tokyo University) which had been cultivated aerobically in the presence of KNO_3 (ref. 4) was mixed with 0.1 M sodium citrate (pH 7.0). After storage at 10° overnight, the suspension was filtered through a Büchner funnel with the aid of celite. To the filtrate, rivanol was added to a final concentration of 0.1%, and the resulting viscous precipitate was separated by filtration. To the filtrate, $(NH_4)_2SO_4$ was added to 100% saturation and the resulting precipitate was collected by filtration. The precipitate was dissolved in a minimum volume of water and the solution was dialysed against 0.01 M phosphate buffer (pH 6.0). The resulting diffusate was charged onto an Amberlite CG 50 column which had been equilibrated with the same buffer as used for the dialysis. Rivanol and some brown impurities were adsorbed on the resin column, while cytochrome remained unadsorbed in the passed solution. To the passed solution was added

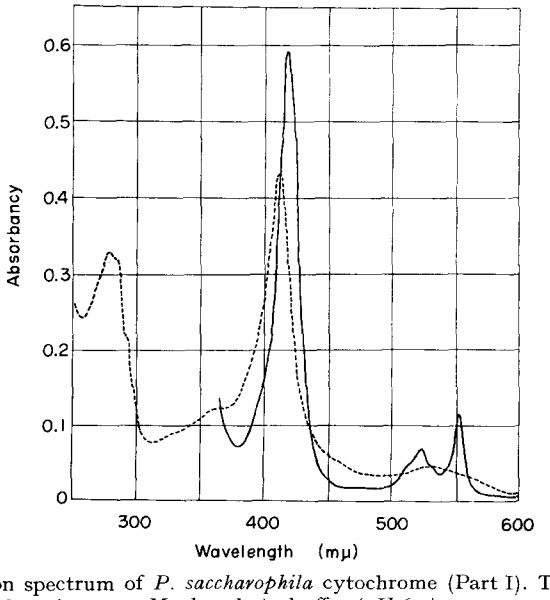

Fig. 1. The absorption spectrum of *P. saccharophila* cytochrome (Part I). The cytochrome preparation was dialysed against 0.04 M phosphate buffer (pH 6.5). - - - -, oxidized; ———, reduced with $Na_2S_2O_4$. Part II showed the same absorption spectrum as Part I.

$(NH_4)_2SO_4$ to 90% saturation and the resulting precipitate was collected by centrifugation for 10 min at 10 000 × *g*. The precipitate was dissolved in a minimum volume of water and dialysed against deionized water overnight. The diffusate was lyophilized and subjected to zone electrophoresis on a potato-starch column at pH 7.0 for 2 days. The cytochrome migrated a considerable distance towards the anode and was separated into a main band (Part I) and a fast-running minor band (Part II). The two bands of the cytochrome were eluted separately from the column and each part was separately subjected to the following procedures. Each was dialysed against 0.01 M phosphate buffer (pH 7.0) and each resulting diffusate was charged on a DEAE-cellulose column which had been equilibrated with the same buffer as used for the dialysis. The cytochrome which was loosely adsorbed on the column was developed with the same buffer as used for the dialysis, and was gradually eluted. The eluate was used as the cytochrome preparation of *P. saccharophila*.

The absorption spectrum of the cytochrome is shown in Fig. 1. It had peaks at 280 mμ, 410 mμ and 530 mμ in the oxidized form, and at 418 mμ, 523 mμ and 552 mμ in the reduced form. The haem of the cytochrome was not extracted with HCl–acetone but split off from the protein moiety by the Ag_2SO_4 method of PAUL[5]. The absorption spectrum of the pyridine haemochromogen of this haem is shown in

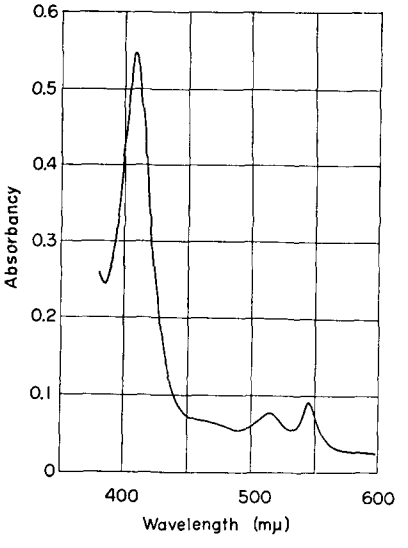

Fig. 2. The absorption spectrum of the pyridine haemochromogen of the haem split off from *P. saccharophila* cytochrome (Part I). To 1.6 ml of the haem solution (pH 11) were added 0.4 ml of pyridine and $Na_2S_2O_4$.

Fig. 2. It had absorption peaks at 409 mμ, 515 mμ and 545 mμ. Therefore, this was haematohaem, and consequently the cytochrome obtained here was a *c*-type cytochrome.

As Figs. 3A and 3B show, the cytochrome reacted very rapidly with Pseudomonas cytochrome oxidase (Pseudomonas cytochrome *c*-551: nitrite, O_2 oxidoreductase)[6] whereas it did not react with cow cytochrome *a* (ref. 7) at all. Thus, the cytochrome was very similar to Pseudomonas cytochrome *c*-551. The E_0' of the cy-

tochrome was determined as +0.237 V at pH 7.0 and 25° using a $K_3Fe(CN)_6$–$K_4Fe(CN)_6$ system[8]. Parts I and II of the cytochrome preparation had the same properties. Thus no cytochrome similar to Pseudomonas cytochrome *c*-554 (ref. 9) was isolated from this organism. Part II may have been produced by a slight modification of Part I or *vice versa*. BONE[10] isolated a *c*-type cytochrome from *P. saccharophila*. However, as the E_0' of this cytochrome is + 0.008 V, his cytochrome is different from the cytochrome described here. Our *P. saccharophila* is a denitrifying bacterium, whereas BONE's organism is a hydrogen-utilizing bacterium.

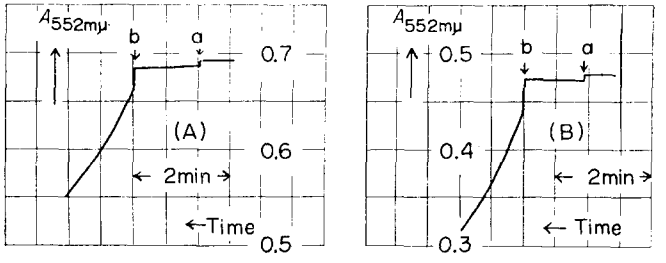

Fig. 3. Oxidation of the reduced form of *P. saccharophila* cytochrome by Pseudomonas cytochrome oxidase and cow cytochrome *a*. The reduced form of the cytochrome was prepared by reduction with $Na_2S_2O_4$ and dialysis against 0.04 M phosphate buffer (pH 6.5). The reaction mixture was composed of 1.5 ml of the cytochrome preparation, 0.03 ml of 5.2 μM cow cytochrome *a* and 0.03 ml of 7.0 μM Pseudomonas cytochrome oxidase. Cow cytochrome *a* and Pseudomonas cytochrome oxidase were added at points a and b, respectively, in the figure. The reactions were carried out at 22.5° and pH 6.5. A, Part I; B, Part II.

It is our intention to test whether the cytochrome described here is identical with the Pseudomonas cytochrome *c*-551 of *P. aeruginosa*, and to examine how Parts I and II of this cytochrome differ from each other.

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