A *b*-type cytochrome from *Streptomyces fradiae*

In a recent survey of the cytochrome composition of various streptomycetes[1] it was noted that in 5 of 13 strains a "*b*"-type cytochrome was the only observable respiratory pigment. Among the organisms of this group, *Streptomyces fradiae* consistently had the most intense absorption bands and its cytochrome was quite amenable to extraction in a soluble form. Since microbial cytochromes of the *b*-type (except yeast cytochrome b_2) have not been studied in a soluble, purified form, purification of the *S. fradiae* cytochrome and studies of the enzyme activities associated with it were undertaken.

Large quantities of cells of *S. fradiae* (Institute of Microbiology Culture No. 3535) were obtained from cultures grown in yeast-extract glucose broth[1] for 72 h at 28° C on a rotary shaker. Cytochrome-containing extracts for enzyme studies were obtained by disrupting a thick washed cell slurry in a 9 KC Ratheyon Sonic Oscillator for 14 min at 10° C. Unbroken cells and large debris were removed by centrifugation for 15 min at 2600 × *g* at 4° C. Cytochrome for purification and spectral studies was obtained by an alkaline extraction method similar to that of NIELANDS[2]. Hyflo supercel (3–3.5 %, w/v) was added to the cells and medium, the mixture was filtered through a Büchner funnel, sufficient distilled water was added to the pads to form a slurry, the pH was adjusted to 10.5 with concentrated NaOH, and the slurry was homogenized in a Waring Blendor for 90 min in the cold (slurry temperature 20–25° C). The alkaline extract was then centrifuged at 34,000 × *g* for 15 min and the supernatant was subjected to $(NH_4)_2SO_4$ fractionation. A 58 % recovery of 8–12-fold purified material was obtained in the 0.55–0.65 saturated fraction. The cytochrome of this fraction was not precipitated by 104,000 × *g* for 45 min and was not dialyzable. Preliminary attempts to effect further purification with Amberlite IRC-50, alumina C γ, Dowex-50 and $Ca_3(PO_4)_2$ gel have not thus far given satisfactory results. Failure to absorb on Amberlite IRC-50 appears to be a property common to microbial cytochromes in general[3,4,5].

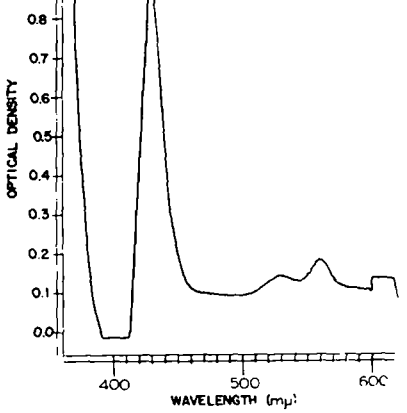

Fig. 1. Difference spectrum of *S. fradiae* cytochrome *b*. An 8-fold purified sample was reduced with $Na_2S_2O_4$ and scanned over the visible range against an oxygenated blank with a Carey Model 14 M recording spectrophotometer. This is a copy of the spectrophotometer tracing.

The cytochrome has the following properties at this state of purity: (1) Absorption maxima: α (reduced): 559 mμ; β (reduced): 530 mμ; γ (reduced): 428 mμ, γ (oxidized): 408 mμ. (Difference spectrum shown in Fig. 1.) Reduced cyanide and pyridine hemochromogens have the same peaks as the reduced cytochrome. (2) It is readily autooxidizable and autooxidizability is not inhibited by KCN, NaN_3, CO, antimycin A or 2-heptyl-4-hydroxy-quinoline N-oxide. (3) It is very stable to alkali as may be inferred from the extraction method, but is readily denatured when the pH is maintained below 6 for several hours.

A general survey of possible reductants of *S. fradiae* cytochrome *b* mediated by sonic extract enzymes revealed that the 559 and 530 mμ bands of reduced cytochrome *b* were visible in the hand spectroscope within 1 min when DPNH, TPNH or $FMNH_2$ were the reductants, and after a 5 min lag with succinate. Lactate and ascorbate were inactive.

Reduced *S. fradiae* cytochrome readily reduced beef-heart ferricytochrome *c* or ferricyanide. That the *b* cytochrome of the extract was the reductant of cytochrome *c* was shown in two ways: (1) The 559 mμ peak of reduced cytochrome *b* disappeared upon addition of ferricytochrome *c* and there was a corresponding increase in absorption at 550 mμ, the peak for ferrocytochrome *c*. (2) When DPNH was added to an evacuated Thunberg tube containing oxidized *S. fradiae* cytochrome *b* and beef heart ferricytochrome *c*, the 559 mμ band appeared within 10 sec whereas the 550 mμ band was not yet visible. After 20 sec a strong 550 mμ band appeared and the 559 mμ band gradually faded. However it is unlikely that a *b* to *c* electron transfer system is functional in *S. fradiae*, since neither cytochrome *c* nor a_3 has been observed in this organism. Furthermore, the *b* cytochrome may not be on the main respiratory pathway of this organism because cyanide does not affect the oxidation and reduction of this pigment but does inhibit growth (100 % at $10^{-3}M$). It is quite possible that cyanide inhibits growth by rendering some other essential enzyme system inactive, or by reducing the availability of some constituent of the medium.

From the limited data available the *Streptomyces* cytochrome appears to be most similar to cytochrome b_5 of liver microsomes[6] and *Cecropia*[7,8]. A more definitive characterization of its properties and physiological role in *S. fradiae* awaits further purification. Such studies are now underway.

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Received March 18th, 1957

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Microsomal cytochrome reductase*

Liver microsomes contain a reductase specific for DPNH and microsomal cytochrome[1]. This enzyme has now been isolated in an essentially homogeneous form from calf liver by a new method. Release of the enzyme from the particles is achieved by the action of snake venom. Further purification is carried out by ammonium sulfate fractionation and column chromatography.

The reductase preparations have a flavo-protein spectrum with absorption maxima in the oxidized form at 273, 390, 461, and 485 mμ. On complete reduction with either excess DPNH or $Na_2S_2O_4$, the absorption peaks at 461 and 485 mμ disappear. The millimolar absorption coefficients at 461 mμ are 10.2 and 2.6 for the oxidized and reduced forms, respectively. Flavin analysis[2], chromatography[3], reactivation of D-amino acid oxidase apoenzyme[4], and reversible splitting of the coenzyme establish that the prosthetic group is flavin adenine dinucleotide. The minimum molecular weight, based on flavin and protein analysis, is 42,000 $\pm$ 2,000. The molecular weight from sedimentation, diffusion and partial specific volume data is 38,400. Complete inactivation of the reductase can be obtained by titration of one essential -SH group with *para*-chloromercuribenzenesulfonate.

Microsomal cytochrome, ferricyanide and indigotetrasulfonate are reduced in the reductase system but cytochrome *c* is inert. Reduction of all three acceptors is competitively inhibited by pyrophosphate.

When the reductase was analyzed for metals by various micro color reactions[5] and by emission spectra, Fe, Mo, Co, Ni, Zn, Cu, Mn, Hg, Pb and Ag were found to be absent. Approximately 2 moles Mg/mole flavin are present.

A detailed report of this work has been submitted for publication.

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Received April 23rd, 1957

* This investigation was supported in part by research grants, H-2768, from the National Heart Institute, Public Health Service, and from the American Cancer Society.