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Purification of cytochrome b_5-like hemoprotein from anaerobically grown yeast

A *b*-type hemoprotein, which is spectroscopically different from the mitochondrial cytochrome *b*, has been reported in anaerobically grown yeast[1,2] and designated cytochrome b_1[3,4]. Although there are several reports[5–10] on this cytochrome, those are confined to the results obtained with whole cells or crude subcellular preparations and it has not yet been well characterized.

This communication reports the purification, some properties and reactivities about the cytochrome which is tentatively called "cytochrome b_{556}".

Yeast, *Saccharomyces cerevisiae*, was cultivated in a medium containing the following per l: 10 g yeast extracts, 5 g peptone, 60 g glucose, 20 mg ergosterol and 4.4 ml Tween 80. The inoculated culture stood for 17 h at 30° in a 5-l flask which was filled to the top with the medium. The cells obtained by the above cultivation contained no aerobic-type cytochromes such as cytochromes *a*, a_3, *b*, *c* and c_1 and contained cytochrome b_{556} and two CO-binding pigments called CO-P-420 and CO-P-450 (ref. 7).

The cells were harvested by centrifugation and washed with cold-distilled water. Washed cells were suspended in 0.1 M potassium phosphate buffer containing 1 mM EDTA, pH 7.5 (Buffer A) (about 100 mg dry cells per ml suspension). After addition of glass beads to the suspension (100 g glass beads per 100 ml suspension), the cells were homogenized in a Waring Blendor for 10 min at top speed under strong refrigeration. After removal of glass beads by decantation, the homogenate was centrifuged at 2100 × *g* for 5 min in order to remove intact cells and nuclear fraction. The supernatant was sonicated (10 kHz, 100 W) for 30 sec and centrifuged at 10000 × *g* for 10 min, and the precipitates which contained few cytochrome b_{556} were discarded. The supernatant was then centrifuged at 79000 × *g* for 90 min to give particulate fraction. The reddish-yellow particulate fraction was resuspended in Buffer A and centrifuged again as above. The washed particulate fraction thus obtained was suspended in 0.05 M potassium phosphate buffer containing 1 mM EDTA, pH 7.5 (Buffer B) (about 15 mg protein per ml suspension). Cytochrome b_{556} and two CO-binding pigments were recovered in this fraction.

In order to solubilize cytochrome b_{556} from the particulate fraction, it was treated with 0.05 % trypsin (EC 3.4.4.4) at 0° for 16 h in Buffer B under anaerobic condition. By this treatment, 60–80 % of the cytochrome was solubilized, but two CO-binding pigments were not solubilized. The solubilized fraction was then applied to a column of DEAE-cellulose equilibrated with Buffer B, and the column was washed with Buffer B and Buffer B containing 0.12 M KCl successively. The adsorbed cytochrome b_{556} was eluted out from the column with Buffer B containing 0.25 M KCl. The cytochrome b_{556}-containing fractions were pooled and concentrated. The concentrated solution was passed through a column of Sephadex G-75 equilibrated with 0.1 M Tris–HCl buffer containing 0.1 M KCl and 1 mM EDTA, pH 7.5. The preparation of cytochrome b_{556} thus obtained was purified about 90-fold over the particulate fraction and the yield was about 15 % from the particulate fraction. The heme content was 4.5–5.5 nmoles/mg protein.

Fig. 1 shows the absorption spectra of oxidized and reduced form of the purified cytochrome b_{556}. The prosthetic group of the cytochrome was determined as protohemin from the shape of absorption spectrum and the position of absorption maxima of the reduced pyridine hemochromogen shown in Fig. 2. The absorption maxima and absorbance indexes calculated from the data of heme analysis are summarized in Table I together with the data of hepatic cytochrome b_5[11]. These spectral properties of cytochrome b_{556} show surprising identity to those of cytochrome b_5.

Among the several reductants tested on the purified cytochrome b_{556}, only sodium hydrosulfide could easily reduced the cytochrome, and other reductants such as sodium borohydride, ascorbate and cysteine reduce it with slow rate only under anaerobic condition. NADH and NADPH could not reduce the purified preparation

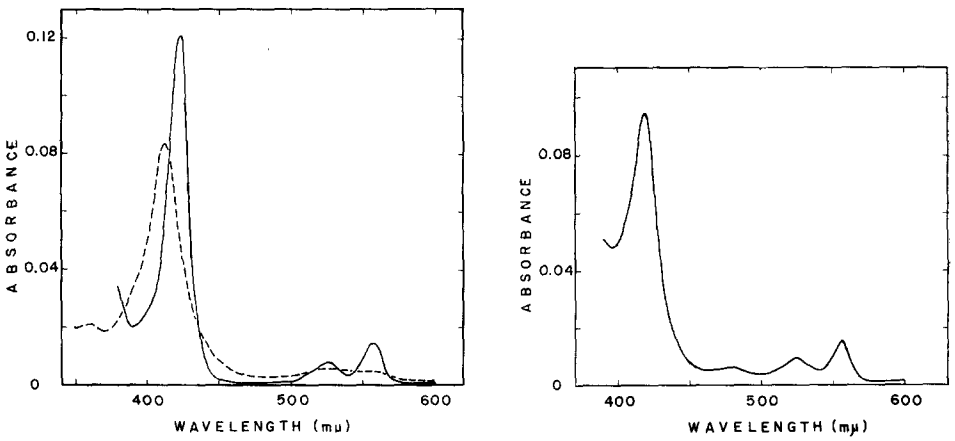

Fig. 1. Absorption spectra of purified cytochrome b_{556}. The preparation purified 86-fold over particulate fraction was used. The preparation was dissolved in Buffer A. The protein concentration was 0.14 mg/ml. – – – –, oxidized form; ———, reduced form with $Na_2S_2O_4$.

Fig. 2. Absorption spectrum of reduced pyridine hemochromogen derived from purified cytochrome b_{556}. The purified preparation of cytochrome b_{556} was dissolved in 0.75 M NaOH containing 10% pyridine and was reduced with a few grain of solid $Na_2S_2O_4$. The protein concentration was 0.09 mg/ml.

TABLE I

SPECTRAL PROPERTIES OF CYTOCHROME b_{556} AND CYTOCHROME b_5

Absorption bands		*Cytochrome* b_{556}		*Cytochrome* b_5**	
		λ_{max} ($m\mu$)	ε_{mM}*	λ_{max} ($m\mu$)	ε_{mM}
Oxidized	Soret	413	117	413	115
Reduced	α	556	24	556	26
	β	525–526	14	526	13
	Soret	423	176	423	171

* Calculated from the data of heme analysis assuming 1 mole of the cytochrome contains 1 mole of protoheme.

** The data of STRITTMATTER[11].

even under anaerobic condition. However, the particulate-bound cytochrome b_{556} was easily reduced by NAD(P)H (Y. YOSHIDA, Y. MORI AND H. KUMAOKA, unpublished observation). This observation suggests the presence of reductase for the cytochrome. The purified cytochrome b_{556} was easily oxidized by air, and the rate of autoxidation was nearly comparable to that of mammalian cytochrome b_5; a tentative value of the first-order velocity constant for autoxidation of the purified cytochrome b_{556} was calculated at 1.04 min^{-1} at room temperature. Among the reactivities of cytochrome b_{556} investigated, the following is noteworthy: purified NADH–cytochrome b_5 reductase (EC 1.6.2.2) from pig liver microsomes could catalyze NADH-linked reduction of the purified cytochrome b_{556} from yeast even with the slower rate than that of reduction of cytochrome b_5. These observations suggest that cytochrome b_{556} resembles to cytochrome b_5 not only on the spectral properties but also on some chemical and enzymatic properties.

From the results described above, we propose that proper nomenclature for the *b*-type cytochrome (cytochrome b_{556}) in anaerobically grown yeast is cytochrome b_5 rather than cytochrome b_1. Cytochrome b_1 is the established name of the cytochrome which has been observed in some bacteria and purified from *E. coli*[12] and the properties of the *b*-type cytochrome in anaerobically grown yeast resembles to cytochrome b_5 in liver microsomes rather than cytochrome b_1 from *E. coli*[12].

Further purification and more detailed characterization of the cytochrome are now in progress.

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