UTILIZATION OF PROTOPORPHYRIN FOR THE SYNTHESIS OF CYTOCHROME C IN YEAST DEFICIENT OF HEME BIOSYNTHESIS

Setsuko Miyake and Takashi Sugimura

Biochemistry Division, National Cancer Center Research Institute

Chuo-ku, Tokyo, Japan

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SUMMARY

The addition of protoporphyrin IX to medium restored respiratory activity of a respiration-deficient mutant of yeast which lacks all hemoproteins. Cytochrome c was purified from the cells grown in medium with ^{14}C-protoporphyrin IX. The specific radioactivity of cytochrome c was identical to that of protoporphyrin IX, implying the utilization of protoporphyrin IX for the synthesis of heme c without endogeneous dilution.

INTRODUCTION

We have previously reported on a respiration deficient (RD) mutant of yeast (Saccharomyces cerevisiae) which lacks all hemoproteins and accumulates coproporphyrin III (1). The phenotype was proved to be a single, recessive gene mutation (cyt) on chromosome (2). The mutant phenotype is temperature sensitive and when cultured at 35°C, it does not synthesize cytochromes, respire or grow on a non-fermentable carbon source (3). However, its capacities to synthesize cytochromes a, b and c, to respire and grow on a non-fermentable substrate are restored by addition of protoporphyrin IX or protohemin IX (3). Thus using this cyt mutant, the synthesis of cytochromes could be investigated. This paper reports the conversion of ^{14}C-labeled protoporphyrin IX, added to the medium, to heme c of cytochrome c without any dilution in the cyt mutant during cultivation at 35°C.

MATERIALS AND METHODS

Z-1 strain (2), a homozygous diploid mutant (cyt/cyt), was used throughout. Cells were cultured aerobically in medium consisting of 2 % proteose peptone No. 3 (Difco), 1 % yeast extract (Difco) and 2 % glucose. Protoporphyrin IX was purchased from Sigma Chemical Co. Respiratory activity of cells was determined polarographically

^{14}C-Protoporphyrin IX was prepared from ^{14}C-δ-aminolevulinic acid with chicken hemolysate by the method of Kawai et al. (4). Its specific activity was 4.2×10^4 cpm/mμmole. ^{14}C-Protoporphyrin IX (specific activity, 630 cpm/mμmole) was added to culture media at a concentration of 5 μg/ml.

Cytochrome c was extracted from cells lysed with ethyl acetate and purified on an Amberlite CG-50 column as described by Sels et al. (5). Electrophoresis of purified ^{14}C-cytochrome c was carried out on a cellulose acetate membrane (Gelman, 2.5 x 17 cm) at 0.5 mA/cm for 3 hr at 0°C in 50 mM ammonium phosphate buffer (pH 6.0). After electrophoresis, densitometric tracing of the cytochrome c band was performed with a Densicord (Model 542, Photovolt) using a 420 mμ filter and after cutting the membrane into 2 mm strips, radioactivity of each strip was counted in a liquid scintillation counter. A tryptic digest of ^{14}C-cytochrome c (1.5 mg) was obtained by the method of Titani et al. (6), and applied to a filter paper (Toyo No. 51A, 60 x 45 cm). The paper was subjected to electrophoresis at 50 V/cm for 70 min, and then descending chromatography for 12 hours. The paper was cut off into 5 cm-wide strips along the direction of chromatography and the radioactivity was determined with a Packard Radiochromatogram Scanner.

RESULTS AND DISCUSSIONS

Fig. 1 shows the restoration of respiratory activity of cyt mutant cells on addition

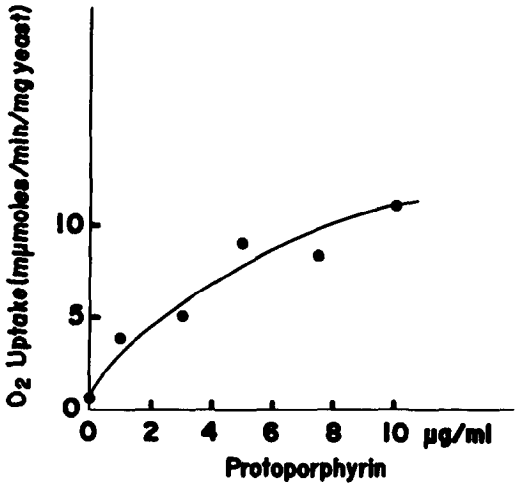

Fig. 1. Effect of addition of protoporphyrin IX on oxygen uptake. Oxygen uptake was measured with a Yanagimoto oxymeter at 30°C in 0.1 M phosphate buffer (pH 5.7) in the presence of 2 % glucose. The abscissa shows the concentration of added protoporphyrin IX in the culture medium.

of various amounts of protoporphyrin IX to the medium. The rate of oxygen uptake increased with increasing concentrations of up to 10 μg/ml of protoporphyrin IX. A concentration of 5 μg/ml was used in subsequent experiments.

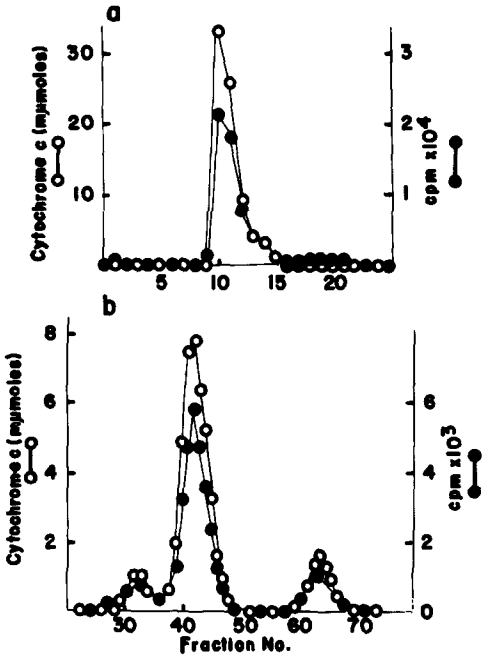

Fig. 2. Elution patterns of crude cytochrome c from Amberlite CG-50 column, (a) 1st column chromatography. After dialysis against 10 mM ammonium phosphate buffer, solubilized cytochrome c was adsorbed to Amberlite CG-50 batch-wise and it was packed to form a small column (1.2 x 10 cm). Cytochrome c was eluted with 0.5 M ammonium phosphate buffer (pH 7.4). Fractions of 1.0 ml were collected. (b) Rechromatography of the above fractions containing cytochrome c on an Amberlite CG-50 column (0.9 x 30 cm). The column was developed with a linear gradient of 0.1 to 0.5 M ammonium phosphate buffer (pH 7.4). Fractions of 1.0 ml were collected.

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cyt Mutant cells were harvested after culture in media with ^{14}C-protoporphyrin IX at 35°C for 48 hr. As shown in Fig. 2a, both peaks of radioactivity and the absorption of oxidized cytochrome c in the Soret region were obtained by one-step elution from Amberlite CG-50 column. Fractions 3 to 8 were pooled, dialysed against 10 mM ammonium phosphate buffer (pH 7.4) and rechromatographed on an Amberlite CG-50 column. Cytochrome c was eluted with linear gradient of 0.1 to 0.5 M

ammonium phosphate buffer (pH 7.4). Cytochrome c was recovered in three distinct peaks (Fig. 2b). The peaks of radioactivity coincided well with the peaks of optical density at 410 mμ. The second and the third peaks seem to be iso-1-cytochrome c and iso-2-cytochrome c, respectively (7). The first peak may be reduced cytochrome c remaining unoxidized during dialysis. The specific radioactivities of the added protoporphyrin IX and the cytochrome c extracted in each peak are given in Table 1. The difference between the specific activities of the cytochrome c isomers and that of the added protoporphyrin IX was within the limits of experimental error.

From these results, it is concluded that the heme c of cytochrome c was formed from exogeneously added protoporphyrin IX without any dilution and de novo synthesis of precursors for heme c was excluded.

To confirm the incorporation of ^{14}C-protoporphyrin IX into the heme c of cytochrome c, the cytochrome c in the second peak shown in Fig. 2b was subjected to electrophoresis on a cellulose acetate membrane in 50 mM ammonium phosphate buffer (pH 6.0). As shown in Fig. 3, the densitometric tracing at 420 mμ coincided well with the pattern of radioactivity obtained by cutting the same membrane into 2 mm strips. The same ^{14}C-cytochrome c preparation used for electrophoresis was also

Table 1

Specific activity of cytochrome c recovered from Amberlite CG-50 column

			Specific Activity cpm/mμmole
Cytochrome c	1st column		711
Cytochrome c	2nd column	peak 1	724
		peak 2	675
		peak 3	785
Added protoporphyrin IX			632

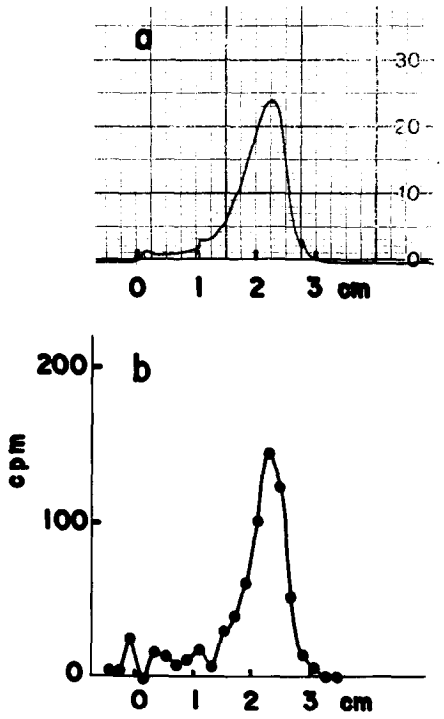

Fig. 3. Densitometric tracing (a) and radioactivity (b) of the ^{14}C-cytochrome c band obtained by electrophoresis. The abscissa shows the distance from the origin.

subjected to tryptic finger print analysis and as shown in Fig. 4, the radioactivity was detected only in the heme peptide region. The remaining portion of the paper exhibited no radioactivity.

These results show that exogeneously added protoporphyrin IX was actually taken up into the cell and converted to the heme c of cytochrome c. Apparently the heme c of cytochrome c was derived entirely from protoporphyrin IX. Sano and Tanaka (8) suggested from in vitro experiments that protoporphyrinogen first combined with the apoprotein of cytochrome c and then iron was incorporated to form heme c. The present results support the utilization of protoporphyrin IX, although the reduction of protoporphyrin to protoporphyrinogen is not completely disproved. Our findings are in good accordance with results showing that cytochrome c synthesis and growth are stimu-

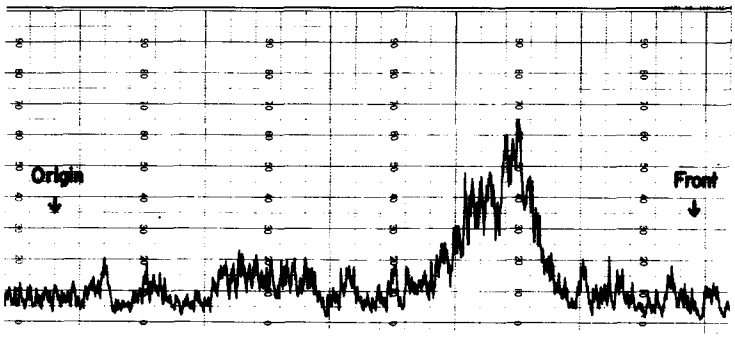

Fig. 4. Radioactivity in the heme peptide obtained by tryptic digestion of ^{14}C-cytochrome c and electrophoresis and chromatography.

lated by addition of protoporphyrin IX to Hemophilus (9,10), Spirillum (11) and E. coli (12). Our preliminary experiments suggested that in the cyt mutant ^{14}C-protoporphyrin was also converted to heme a. The prosthetic group of hemoproteins may be important in regulating the biosynthesis of their protein components, so this cyt mutant will be useful in analysis of the mechanism of biosynthesis of cytochromes.

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