Changes in microsomal hemoprotein content and glucose 6-phosphatase activity during low-temperature acclimation of *Tetrahymena pyriformis*

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To examine the temperature-associated alterations in microsomal enzymes in an eukaryote, *Tetrahymena* cells were transferred from 39.5–15°C. The content of cytochrome *b*-560$_{ms}$, a cytochrome similar to but not identical with liver microsomal cytochrome b_5, was slightly increased within 2 h after temperature shift-down, then gradually decreased, and reached the cytochrome *b*-560$_{ms}$ level of the 15°C-isothermally grown cells within 10 h following the temperature-shift. Cytochrome P-450 was not detected in *Tetrahymena* microsomes. The presence of a co-binding hemoprotein with a main peak at 420 nm was, however, observed. The amount of this hemoprotein was unchanged for 2 h after cold-shift. Later, it increased gradually and reached the maximum level at 10 h. Glucose 6-phosphatase activity increased slightly at 10 h, when cell division had already been regained. We conclude that cytochrome *b*-560$_{ms}$, which is linked to the desaturase, may be essential for short-term adaptation (2 h after shift), while the amount of co-binding hemoprotein and the activity of glucose 6-phosphatase would be associated with long-term adaptation (10 h after temperature shift) in *Tetrahymena* cells.

Tetrahymena *Microsome* *Hemoprotein* *Temperature acclimation* *Glucose 6-phosphatase*

1. INTRODUCTION

The poikilothermic organisms adjust the fatty acid composition of their phospholipids in response to growth temperature [1–3]. This regulatory mechanism leads to a change in the temperature of the order–disorder lipid phase transition and makes membrane function at lower temperatures possible [4].

In *Escherichia coli*, β-ketoacyl-ACP synthase II of the fatty acid biosynthetic pathway is required for regulating the fluidity of cell membranes in response to low temperature [5]. However, unlike in prokaryotic cells, in a eukaryotic organism, *Tetrahymena pyriformis*, the desaturation system which requires O_2 and NAD(P)H, may play an important role during low temperature acclimation [6]. In [7,8] the induced activities of *Tetrahymena* desaturase enzymes were found to be maximal 2 h after temperature shift-down (39.5–15°C) and thereafter declined to the initial levels [7,8]. In contrast, the activities of *Tetrahymena* lysosomal enzymes, such as acid phosphatase and protease, were increased 8 h after temperature shift [9].

Although we have demonstrated the low-temperature-associated alterations in reductase and desaturase activities in *Tetrahymena* microsomes [10], nothing is as yet known regarding changes in the hemoprotein content of the microsomal electron transport system during temperature acclimation. In addition, as lipids are required for the activity of microsomal glucose 6-phosphatase (G-6-Pase) [11], we also focused our attention on the alteration of the activity of

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this marker enzyme in the endoplasmic reticulum.

These experiments were undertaken to determine whether changes in microsomal membrane-bound enzymes, such as hemoproteins and G-6-Pase, occur during a cold temperature shift (39.5–15°C).

2. MATERIALS AND METHODS

2.1. *Growth of organism and isolation of microsomal fraction*

Cells of the thermo-tolerant strain NT-1 of *Tetrahymena pyriformis* were grown at 39.5°C in an enriched proteose–peptone medium [12]. The culture in the logarithmic phase ($50–60 \times 10^4$ cells/ml) was cooled to 15°C over a 30 min period (0.8°C/min), and cells were harvested after the indicated incubation periods at 15°C. The microsomal fraction was isolated from homogenates prepared with phosphate buffer (0.2 M K_2HPO_4, 0.2 M KH_2PO_4, 3 mM EDTA and 0.1 M NaCl, pH 7.4) as in [13]. The collected microsomes were washed once in fresh 0.1 M Tris–HCl buffer (pH 7.6). Protein was determined as in [14].

2.2. *Hemoprotein contents*

Tetrahymena microsomal cytochrome *b*-560_{ms} was reduced by adding 100 nmol NADH, and its amount was determined by measuring the reduced-minus-oxidized difference spectrum, using the difference of absorbance of the cytochrome between 425 and 410 nm as 216 $mM^{-1}.cm^{-1}$ [15,16]. The amount of co-binding hemoprotein in the microsomes was measured in the conventional way; $Na_2S_2O_4$ was added to both cuvettes and carbon monoxide to the sample cuvette, and the difference spectra were recorded. The peak heights of absorption maxima at 420 nm were determined as the distances (in absorbance units) of the respective absorbance peaks from the baseline.

2.3. *Enzyme assays*

G-6-Pase activity was measured according to the modified method of Ames [17]. The incubation mixture, in a 0.5 m final volume, contained the following: 0.1 M sodium acetate–0.1 M sodium succinate buffer (pH 6.0) and 60 mM glucose 6-phosphate. The reaction was initiated by the addition of microsomal fraction (0.6–2.8 mg protein) after preincubation for 1 min. Incubations were carried out for 10 in at 39°C. The reaction was stopped by adding 3 ml 10% ascorbic acid–42% ammonium molybdate in 1 N H_2SO_4 (1:6, v/v) solution. The absorbance was measured at 660 nm. The specific activity of the phosphase was calculated as nmol phosphorus released. min^{-1}.mg microsomal $protein^{-1}$.

3. RESULTS AND DISCUSSION

The cytochrome composition of *Tetrahymena* cells has been described in [18]. Although there was no significant hydroxylation of xenobiotics in *Tetrahymena* microsomes [19], NAD(P)H-dependent fatty acyl-CoA desaturase activities were observed in microsomes [6]. Moreover, we have isolated cytochrome *b*-560_{ms} [16] (previously called *Tetrahymena* cytochrome b_5) [15], which is similar to mammalian cytochrome b_5, and NADPH-cytochrome *c* reductase [20] from *Tetrahymena* microsomes. The relative molecular masses of these enzymes are about 22000 (cytochrome *b*-560_{ms}) and 70000 (NADPH-cytochrome *c* reductase) as determined by SDS–PAGE.

In general, in liver cells a remarkable increase in

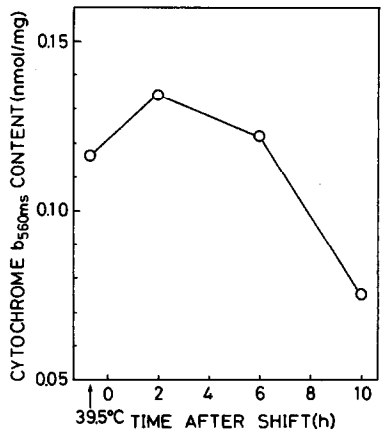

Fig. 1. Changes in the amount of cytochrome *b*-560_{ms} in microsomes from *Tetrahymena* cells after temperature-shift from 39.5°C–15°C. Microsomes were isolated from cells harvested after the indicated incubation periods at 15°C. Concentrations of cytochrome *b*-560_{ms} were expressed in nmol/mg protein. Each point indicates mean values from 3 separate experiments.

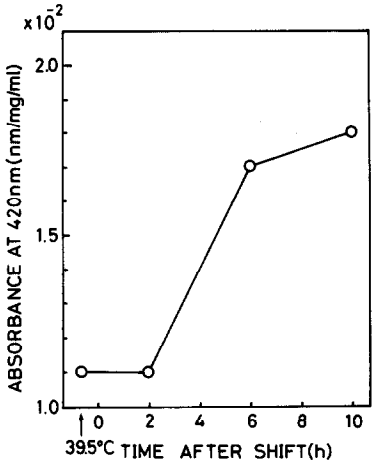

Fig. 2. Changes in the amounts of co-binding hemoprotein in microsomes from *Tetrahymena* cells after temperature-shift from 39.5–15°C. Microsomes were isolated from cells harvested after the indicated incubation periods at 15°C. The peak height of absorption maxima at 420 nm were determined as the distance from the respective absorbance peaks to the baseline. Each point indicates mean values from 3 separate experiments.

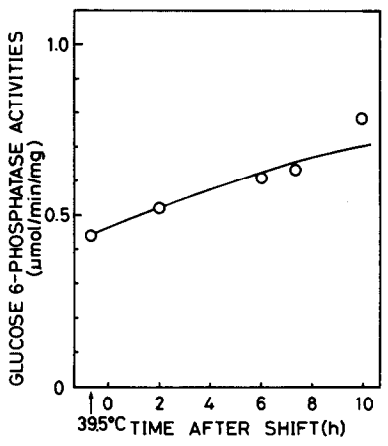

Fig. 3. Changes in G-6-Pase activity in microsomes from *Tetrahymena* cells after temperature shift from 39.5–15°C. Microsomes were isolated from cells harvested after the indicated incubation periods at 15°C. Each point indicates mean values from 3 separate experiments.

desaturase activity has been observed during hibernation, without a corresponding increase in the activity of the microsomal electron-transport chain [21]. In contrast, when *Pseudomonas aeruginosa* cells grown at 46°C were transferred to 37°C, a sudden burst in the synthesis of all cytochromes, such as a_1, *b*, and *c*, was experienced [22]. To examine the temperature-associated alterations of microsomal hemoproteins in a eukaryote, *Tetrahymena*, the temperature of the cultures was shifted from 39.5°C–15°C. The cytochrome *b*-560_{ms} content was slightly increased within 2 h after temperature shift-down, then gradually decreased and reached the level of the isothermally (15°C) grown cells within 10 h (fig. 1).

Unexpectedly, cytochrome *P*-450 was not detected in *Tetrahymena* microsomes by low temperature spectroscopy [18]. However, co-binding hemoprotein, with a main peak at 420 nm, was observed in the microsomes [18,23]. The temperature-associated alterations in the level of this hemoprotein are shown in fig. 2. Although the level of this hemoprotein remained steady for 2 h after cold-shift, later it started to increase gradually. After 10 h incubation at 15°C, the level of co-binding hemoprotein was identical to that found in the microsomes from cells grown at 15°C. Although the function of co-binding hemoprotein in *Tetrahymena* microsomes is obscure, in-

Table 1

Alterations in *Tetrahymena* enzyme activities after temperature shift (39.5–15°C)

	39.5°C-grown cells (%)	Time after shift		
		2 h (%)	6 h (%)	[Ref.]
Palmitoyl-CoA desaturase	100	433	60	[7]
Stearoyl-CoA desaturase	100	283	43	[7]
NADH-ferricyanide reductase	100	153	123	[7]
NADH-cytochrome *c* reductase	100	219	132	[7]
NADPH-cytochrome *c* reductase	100	149	88	[7]
b-560_{ms}-oxidation	100	368	112	[22]
Protease	100	206	1816	[6]
Acid phosphatase	100	118	136	[6]

terestingly, the amount of this hemoprotein increased during low temperature acclimation.

To gain a deeper insight into the effect of temperature on functions associated with the microsomal membrane, we chose for further studies G-6-Pase a typical marker enzyme of endoplasmic reticulum. The alterations in G-6-Pase activity after temperature shift-down are illustrated in fig. 3. Interestingly, the G-6-Pase activity increased slightly for 10 h and exhibited no peak 2 h after shift-down. Consequently, although G-6-Pase activity appears to be controlled by the physical state of membrane lipids [24,25], it does not seem to be affected during the short-term adaptation period after temperature shift-down.

Table 1 summarizes the alterations of *Tetrahymena* enzymes after temperature shift (39.5–15°C). It may be seen that the activity of electron-transport enzymes involved in fatty acid desaturation increased within 2 h after the shift. In contrast, the level of co-binding hemoprotein, and the activity of G-6-Pase, protease and acid phosphatase gradually increased without the 2 h peak. *Tetrahymena* cells were able to grow and divide within about 10 h after temperature shift-down, when the distribution of membrane particles, as examined by freeze-fracture electron microscopy, was restored to normal [7].

These data suggest that the microsomal electron-transport enzymes which participate in fatty acid desaturation are essential for short-term adaptation (2 h after shift), whereas changes in the activity of G-6-Pase and in the amount of co-binding hemoprotein appear to be associated with long-term adaptation (10 h after shift), when cell division has already been regained.

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