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EFFECTS OF STEROL MANIPULATION ON MICROSOMAL DESATURASE ACTIVITIES IN TETRAHYMENA: WITH REGARD TO THERMAL ACCLIMATION

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SUMMARY: There was a great increase in microsomal palmitoyl-CoA desaturase activity of ergosterol-replaced Tetrahymena (ergosterol-cells), which exhibited a pronounced elevation of palmitoleate ($16:1^{\Delta 9}$) in fatty acid composition. At 2 hr after the growth temperature-shift from 34 to 15°C (shift-down), palmitoyl-CoA desaturase activity in ergosterol-cells increased 6-fold compared to that in native cells containing tetrahymanol before the shift-down. These results suggest that, unlike drastic increases of palmitoyl-CoA, stearoyl-CoA and oleoyl-CoA desaturase activities by the shift-down in native cells, ergosterol-cells accomplish an adaptive modification of fatty acid composition by a preferential increase in palmitoyl-CoA desaturase activity, being which is principally due to the increased content of the terminal component (cyanide sensitive factor; CSF) of the desaturase system.

INTRODUCTION: One useful approach for a better understanding of the involvement of lipids in membrane functions is to manipulate the lipid composition of membrane by supplementation with various compounds. The earlier studies demonstrated that alterations of lipid composition induced by numerous supplements influence the physical properties and enzyme activities of membranes in many organisms (1-3). As reported in our previous papers (4, 5), a unicellular eucaryote, Tetrahymena pyriformis, can undergo in vivo manipulation of membrane lipids in response to exogenous ergosterol. In addition, we have also employed this cell as a useful system to gain insight into the molecular mechanism(s) of temperature acclimation of membrane lipids (6-8).

In the present study we investigated changes induced by the ergosterol replacement in the activities of microsomal desaturation and electron transport enzymes, and also their activities in sterol modified cells exposed to temperature shift-down from 34 to 15°C.

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MATERIALS AND METHODS:

Cell growth and isolation of microsomes: Ergosterol was added to the culture medium pre-warmed to 60°C as a sterile ethanolic solution prior to incubation to a final concentration of 2 mg/200 ml of culture (4). A thermotorelant strain NT-1 of T. pyriformis was grown at 34°C for 24 hr in an enriched proteose peptone medium with or without ergosterol (9). As a native culture containing tetrahymanol, 0.2 ml of absolute ethanol was added to the medium. Both types of culture at the mid-exponential phase were cooled to 15°C over 30 min, and cells were harvested after the indicated incubation periods at 15°C. Microsomes were isolated from homogenates prepared in 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 previously described (10). Collected microsomes were washed once by suspending in 0.1 M potassium phosphate buffer (pH 7.4). Protein was determined by the method of Lowry et al. (11).

Lipid composition: Lipids were extracted from whole cells by the method of Bligh and Dyer (12). Fatty acid methyl esters prepared with BF_3 according to the method of Morrison and Smith (13), were analyzed by gas-chromatography as previously described (14).

Enzyme assays: The activities of palmitoyl-CoA, stearoyl-CoA and oleoyl-CoA desaturases were assayed using 20 nmol of [^{14}C] palmitoyl-CoA, [^{14}C] stearoyl-CoA and [^{14}C] oleoyl-CoA, respectively, 50 nmol of NADH, 0.1 M potassium phosphate (pH 7.2), 100-400 µg of microsomes in a final volume of 0.5 ml at 34°C as described before (8).

The NAD(P)H-cytochrome c and NADH-ferricyanide reductase activities were determined using 20 nmol of cytochrome c or 500 nmol of potassium phosphate, 100 nmol of NADH or NADPH, 20-100 µg of microsomes, and 0.1 M potassium phosphate buffer (pH 7.4) in a total volume of 1.0 ml at 34°C (8). The activities were calculated using respective extinction coefficients of 19.6 $mM^{-1} \cdot cm^{-1}$ (15) and 1.02 $mM^{-1} \cdot cm^{-1}$ (16).

CSF (terminal component) activity was assayed by measuring spectrophotometrically the palmitoyl-CoA-stimulated reoxidation rate of NADH-reduced cytochrome b_5 by monitoring the change in absorbance difference between 425 and 410 nm. The sample cuvette contained microsomes with 0.2 nmol of cytochrome b_5, 200 nmol of fresh Na_2S to inhibit mitochondrial cytochrome oxidase, and 0.1 M Tris-HCl buffer (pH 7.2) in a final volume of 3.0 ml. The rate of reoxidation of cytochrome b_5 reduced by 2 nmol of NADH at 34°C was calculated according to the procedure of Oshino and Sato (17) with a slight modification (18).

Chemicals: The following chemicals were obtained from commercial sources: [1-^{14}C] palmitoyl-CoA, [1-^{14}C] stearoyl-CoA and [1-^{14}C] oleoyl-CoA (New England Nuclear Corp., Boston, MA); palmitoyl-CoA, stearoyl-CoA and oleoyl-CoA (P-L Biochemicals, Inc., Milwaukee, WI); NADH, NADPH, horse heart cytochrome c and ergosterol (Sigma Chemical Co., St. Louis, MO); potassium ferricyanide (Kishida Chemical Co., Ltd., Osaka). Other chemicals were of the highest purity available from commercial sources.

RESULTS AND DISCUSSION: Manipulation of membrane lipid composition has been applied to many types of cells (19-22), either procaryotic or eucaryotic. In an earlier study, we showed that Tetrahymena can replace the native tetrahymanol with ergosterol by adding the sterol in the culture medium (4). In addition, it was found that ergosterol-replaced membranes became less fluid, when compared with the native membranes, as examined by fluorescence polarization (5).

Table I Changes in fatty acid composition of total lipids of native and ergosterol-cells during temperature shift-down from 34 to 15°C

Fatty acids	Native cells			Ergosterol-cells		
	34°C	34 to 15°C		34°C	34 to 15°C	
	(n=6)	2 hr (n=3)	6 hr (n=3)	(n=6)	2 hr (n=3)	6 hr (n=3)
12:0	2.6±0.4	1.0±0.1	0.8±0.1	2.5±0.2	1.4±0.1	1.8±0.1
14:0	9.6±0.7	8.1±0.4**	7.2±0.7*	14.8±0.1*	8.8±0.7#	8.5±0.2#
16:0	12.1±0.4	7.5±0.6*	7.5±0.9*	7.1±0.2*	6.6±0.6	5.9±0.7#
$16:1^{\Delta^9}$	13.6±0.8	18.1±0.9*	15.7±0.8*	20.0±0.5*	22.2±1.1#	20.9±0.6
18:0	3.3±0.4	4.5±0.8	4.4±0.5	4.6±0.2*	4.4±0.3	4.3±0.2
$18:1^{\Delta^9}$	5.6±0.6	5.8±0.3	7.0±0.6	4.5±0.2*	4.8±0.5	4.4±0.5
$18:2^{\Delta^{6,11}}$	3.4±0.4	3.9±0.4	4.9±0.3*	4.1±0.2*	4.8±0.6	5.3±0.4#
$18:2^{\Delta^{9,12}}$	14.4±0.8	12.7±2.1	12.2±1.7	9.4±0.3*	11.0±0.6	10.7±0.8
$18:3^{\Delta^{6,9,12}}$	23.9±1.2	26.5±1.6	27.9±0.8*	20.2±1.4*	24.6±2.6	25.0±1.6#
$16:1^{\Delta^9}$ /16:0	1.1	2.4	2.1	2.8	3.4	3.5
U/S ratio	2.2	3.2	3.4	2.0	3.1	3.2

Details for the quantitative analysis of fatty acid composition are described in the MATERIALS AND METHODS. Values are averages ± S.D. of 3-6 independent experiments. U/S ratio, the ratio of major unsaturated to major saturated fatty acids. n, the number of experiments performed. *, $P<0.01$; **, $P<0.02$ compared to native cells. #, $P<0.01$ compared to ergosterol-cells before a shift.

In the present study, ergosterol-cells were found to show reproducible and significant changes in fatty acid composition; great increases in myristate (14:0) and palmitoleate ($16:1^{\Delta^9}$) accompanied by significant decreases in long-chain unsaturated fatty acids (oleate; $18:1^{\Delta^9}$, linoleate; $18;2^{\Delta^{9,12}}$, γ-linolenate; $18:3^{\Delta^{6,9,12}}$) (Table I). These changes are expected to be due to the increased activity of palmitoyl-CoA desaturase involving the palmitoleate pathway (16:0 → $16:1^{\Delta^9}$ → $18:1^{\Delta^{11}}$ → $18:2^{\Delta^{6,11}}$), and to the decreased activities of stearoyl-CoA and oleoyl-CoA desaturases in the stearate pathway (18:0 → $18:1^{\Delta^9}$ → $18:2^{\Delta^{9,12}}$ → $18:3^{\Delta^{6,9,12}}$) (Fig. 1), and would also result from the decrease of chain elongation. This altered fatty acid composition may result in decreased membrane fluidity in ergosterol-cells. Therefore, ergosterol-cells accomplish an adaptive modification by increasing formation of palmitoleate against exogenous ergosterol via a preferential enhancement of palmitoyl-CoA desaturase activity. This was reflected as the increase in the ratio of palmitoleate to palmitate (16:0) (1.1 → 2.8) in ergosterol-cells compared to native cells containing tetrahymanol (Table I). In contrast, stearoyl-CoA and oleoyl-CoA desaturase activities decreased in ergosterol-cells (Fig. 1). Blank et al.

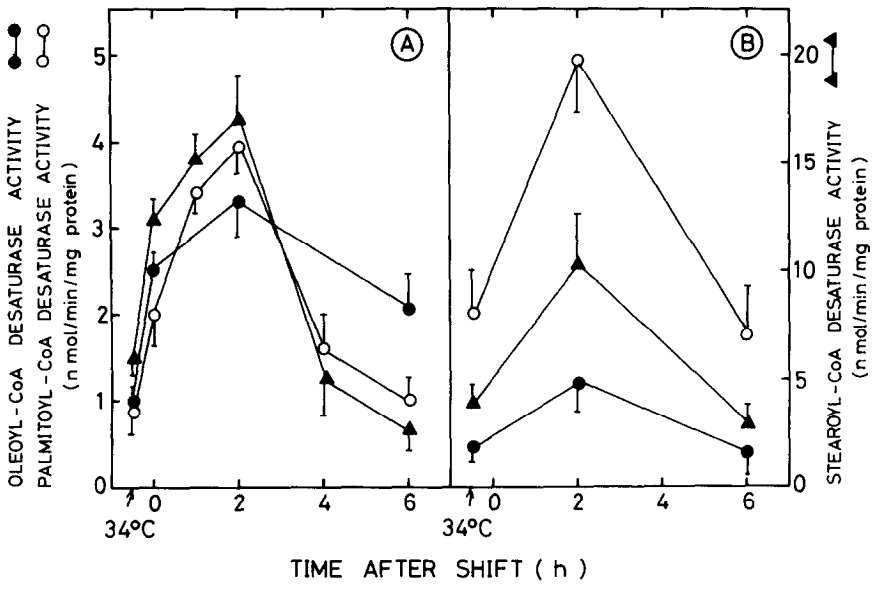

Fig. 1 Changes in activities of palmitoyl-CoA, stearoyl-CoA and oleoyl-CoA desaturases in microsomes from native and ergosterol-cells following the shift 34 to 15°C. Details for the enzyme assays are described in the MATERIALS AND METHODS. Values are averages ± S.D. of 3-6 independent experiments, each performed in duplicate. o——o, palmitoyl-CoA desaturase; ▲——▲, stearoyl-CoA desaturase, ●——●, oleoyl-CoA desaturase. A, native cells, B , ergosterol-cells.

(20) have demonstrated that there was a decrease in stearoyl-CoA desaturase activity in microsomes of L-M cells of which membrane phospholipid polar headgroup composition was modified by the treatment with N-isopropylethanolamine, and have suggested that the reduced desaturase activity would result from altered membrane fluidity in the lipid modified L-M cells.

When native and ergosterol-cells were shifted to 15°C, on the other hand, the proportion of palmitoleate reached the maximum level, whereas linoleate and γ-linolenate gradually increased (data not shown), with being represented by the increased U/S ratio of unsaturated (U) to saturated fatty acids (S). Within 2 hr after the shift-down of native cells, all three fatty acyl-CoA desaturase activities rapidly increased 3-4 times compared to 34°C-native cells (Fig. 1). There was, however, a progressive increase up to 6-fold in palmitoyl-CoA desaturase activity at 2 hr after a shift-down of ergosterol-cells compared to that of 34°C-native cells, and 2.5-fold to that

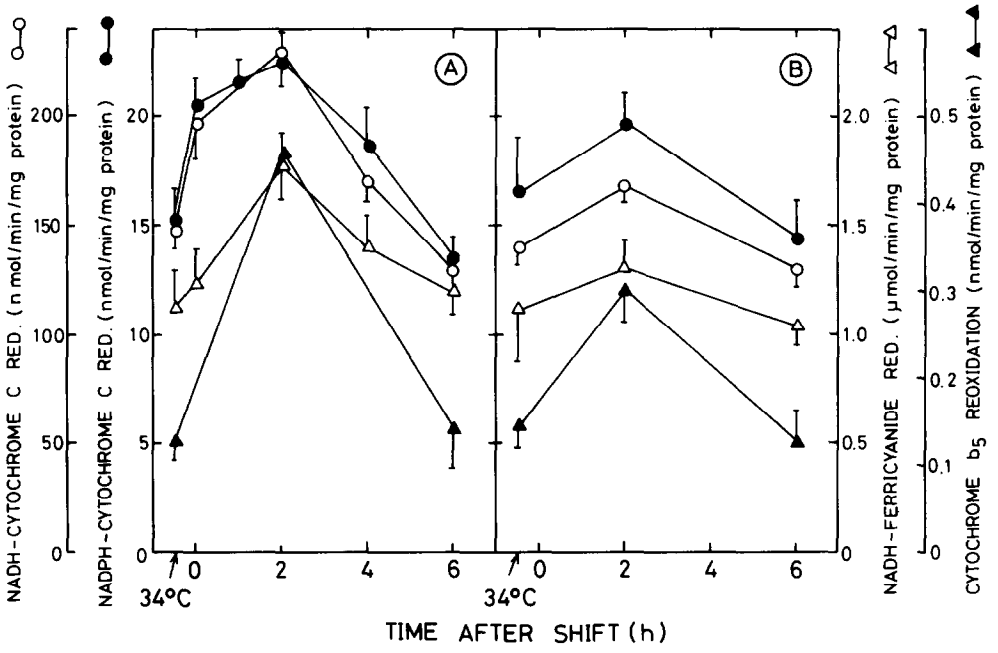

Fig. 2 Changes in activities of CSF (terminal component) of the desaturase system and three reductases in microsomes from native and ergosterol-cells following the shift 34 to 15°C. Details for the enzyme assays are described in the MATERIALS AND METHODS. Values are averages ± S.D. of 3-6 independent experiments. ○——○, NADH-cytochrome c reductase; ●——●, NADPH-cytochrome c reductase; △——△, NADH-ferricyanide reductase. ▲——▲, CSF activity. A, native cells; B, ergosterol-cells.

of 34°C-ergosterol-cells. In addition, the shift-down-induced enhancement of the lowered activities of stearoyl-CoA and oleoyl-CoA desaturases caused by ergosterol-replacement is required to make membranes more fluid during thermal adaptation of ergosterol-Tetrahymena membrane lipids. These results indicate that, when ergosterol-cells with less fluid membranes were exposed to a lower temperature, there was a further accumulation of palmitoleate due to the increased palmitoyl-CoA desaturase activity.

On the other hand, the activities of NAD(P)H-cytochrome c and NADH-ferricyanide reductases, unchangeable in 34°C-ergosterol-cells compared to 34°C-native cells, were slightly increased by the shift-down. Nevertheless, CSF activity increased over 2-fold at 2 hr after a shift-down (Fig.2), indicating that the increases of overall desaturase activities in ergosterol-cells may be due principally to the increased content of the terminal component (cyanide sensitive factor; CSF) of the desaturase system.

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