Regulation of Fatty Acid Unsaturation in Encysting Acanthamoeba Cells

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Abstract. The degree of fatty acid unsaturation and average chain length are closely similar for microsomal membranes from exponential-phase trophozoites and cysts of Acanthamoeba castellanii despite significant differences in fatty acid composition. The same trend was apparent for total fatty acids extracted from whole cells. The observations suggest that the organism regulates these lipid parameters during differentiation in order to maintain optimum membrane lipid viscosity, and are consistent with previous electron spin resonance measurements indicating that the fluidity of microsomal membranes does not change during encystment. About 75% of the microsomal fatty acids are unsaturated for both cysts and amoebae. Wide-angle X-ray diffraction of phospholipid liposomes prepared from lipid extracts of the membranes has indicated that this high level of unsaturation renders the phospholipid exclusively liquid-crystalline at temperatures as low as 9°C for rough microsomes and −1.5°C for smooth microsomes. Thus, by retaining a high proportion of unsaturated fatty acids throughout its differentiation cycle, the organism gains some protection in its natural soil habitat against lateral phase separation of membrane lipids.

The soil amoeba Acanthamoeba castellanii assumes two states of differentiation—an amoeboid trophozoite and a resting cyst. In suspension cultures, trophozoite numbers increase exponentially until limiting cell densities are reached in the stationary phase. Trophozoites respond to adverse environmental conditions by encysting, and stationary-phase cultures encyst spontaneously but not to completion [25]. Synchronous encystment can be achieved by placing trophozoites into nutrient-free medium [18]. Mature cysts are dormant cells, virtually insensitive to stress, and can withstand prolonged starvation, dessication, and extremes of temperature [1,18].

Growth deceleration and encystment bring about marked changes in metabolism and macromolecular synthesis [5]. The properties of membranes are also altered. For example, there is a proliferation during the transition to stationary phase of cytoplasmic membranes that are broken down as the cells encyst [3,6,19], and microsomal enzyme activities are very much lower in mature cysts than in trophozoites [19]. The activities of membrane-bound enzymes are known to be sensitive to the composition and physical state of membrane lipids [11]. However, we have previously shown that at the growth temperature (29°C) there is no consistent change in the fluidity of bulk membrane lipid that could account for the observed attenuation in the activities of the microsomal enzymes during encystment [20]. In the present study, we report that this constancy of membrane microviscosity is in part attributable to regulation of fatty acid unsaturation and chain length, parameters known to influence phospholipid microviscosity.

Materials and Methods
Trophozoites of Acanthamoeba castellanii (Neff) were cultured axenically at 29°C in 2-liter Erlenmeyer flasks containing 1 liter of medium [22]. The flasks were inoculated with 2 ml of stationary-phase culture and maintained for 40 h to obtain exponential-phase cells and for 7 days to obtain cells in stationary phase. Encystment was induced by transferring stationary-phase cells to nutrient-free medium [7]. Rough and smooth microsomal membranes were isolated as described previously [19].

Lipids were extracted from pellets of washed whole cells and the isolated membrane fractions with chloroform/methanol (2:1, vol/vol) and partitioned against 0.7% NaCl as described by Folch, Lees, and Sloane-Stanley [10]. Fatty acid methyl esters were prepared from the total lipid extract by transesterification in methanolic BF3 (14% BF3 in methanol) [17] and analyzed by flame-ionization gas-liquid chromatography. A stainless steel column (182 × 0.32 cm ID) containing 10% diethylene glycol succinate stabilized on 80/100 mesh chromosorb W and maintained at 170°C was used for the analysis.

Phospholipids were purified from the total lipid extracts by silica-gel chromatography [21]. Approximately 50 mg of lipid in chloroform solution was applied to a column of silica gel 60 (1.1 ×
Results and Discussion

Fatty acids of whole cells. The fatty acid profile of whole amoebae is altered considerably during growth deceleration and encystment (Table 1). Our results indicate that there is a shift towards the terminal whole amoebae is altered considerably during growth

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Table 1 shows that cysts have significantly higher concentrations of 18:2 (linoleic acid) but lower con-