Influence of plasma membrane fluidity on phagocytic activity in Acanthamoeba castellanii

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Adaptation of biological membrane composition is considered to be a key factor in allowing continued physiological function of organisms under varying external conditions [1,2]. The membrane lipid composition of the soil amoeba Acanthamoeba castellanii changes markedly during growth [3] and at low temperature [4,5]. While rates of cell division and respiration of A. castellanii appear to be unaffected by these changes, a possible relationship with phagocytosis has been suggested [5]. In this study we confirm that temperature-dependent changes in phagocytosis correlate with changes in plasma membrane composition and fluidity.

Chilling of early stationary phase (48 h) A. castellanii cultures from 30°C to 15°C resulted in an increase in the whole cell fatty acid unsaturation index from approximately 1.76 to 2.18 within 12 h. Similarly, while rates of phagocytosis of latex beads [6] were decreased by approximately 98% on chilling, after 12 h at 15°C the rate had recovered to approximately 27% of its value at 30°C. Such changes were not evident following chilling of mid-exponential phase cells, where fatty acid composition is not influenced by temperature shift [5].

Plasma membrane fractions [4] were isolated from cells previously incubated at 30°C and 15°C. As in whole cells, the degree of fatty acid unsaturation of plasma membranes increased markedly at 15°C. This effect was most evident in the linoleate to oleate ratio which increased from about 0.2 to 3.0 after 12 h incubation at 15°C. Differential scanning calorimetry revealed endotherms with peaks at about 49°C and 34°C in lipid extracts from 30°C- and 15°C-acclimated membranes, respectively. The low enthalpies and high temperatures of these transitions were consistent with the formation of non-lamellar phases, probably of phosphatidylethanolamine which constituted ~50% of plasma membrane phospholipids. Endotherms associated with gel to liquid-crystalline transitions may have been masked by the presence of sterols or neutral lipids in lipid extracts [7].

Steady state fluorescence depolarisation studies of plasma membrane fractions were performed using 1,6-diphenyl 1,3,5-hexatriene (DPH) as a probe of bulk phase fluidity [8]. Fluorescence anisotropy in membrane samples studied with decreasing assay temperature, suggesting reduced freedom of motion of the probe (Fig. 1a). Breaks in the slopes of derived plots, which are generally interpreted as gel to liquid-crystalline phase transitions [8], occurred at ~24.5°C and 17.5°C in 30°C- and 15°C-acclimated membranes, respectively. Homeoviscous and homeophasic efficacies [1,2], calculated from these plots, were approximately 22% and 47%, respectively.

Short-term (10 min) rates of phagocytosis decreased with assay temperature. Arrhenius plots of phagocytotic activity also revealed breaks in derived slopes which occurred at approximately 25°C and 17.5°C in cells acclimated to 30°C and 15°C, respectively. These temperatures correlated very well with those obtained by fluorescence depolarisation.

The results suggest that an increase in the relative degree of unsaturation of plasma membrane lipids, following chilling of A. castellanii, results in a downward shift in the gel to liquid-crystalline phase transition temperature. These transition temperatures coincided with abrupt changes in the temperature-dependence of phagocytosis, clearly suggesting a relationship between membrane fluidity and phagocytosis. Indeed, the present results indicate that measurement of phagocytotic rates may represent a novel method for estimating phase transition temperatures in phagocytic cells.

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