ALTERATIONS OF CHARACTERISTIC TEMPERATURES FOR LECTIN INTERACTIONS IN LM CELLS WITH ALTERED LIPID COMPOSITION

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SUMMARY. Alteration of the fatty acid composition of mouse LM cell lipids dramatically affected the concanavalin A binding and concanavalin A-mediated hemadsorption properties of these cells. A critical temperature for these two concanavalin A related phenomena observed at 15-19° in cells with unaltered fatty acid composition was shifted to 22-28° for cells containing a higher proportion of saturated fatty acids and lowered to 7-11° for cells containing polyunsaturated fatty acids substituted for monoenoic unsaturated fatty acids. In contrast, a second critical temperature (at 5-7°) observed for concanavalin A binding and concanavalin A-mediated hemadsorption to LM cells was essentially unchanged by alterations in cellular lipid fatty acid composition. We conclude that a change in membrane lipid freezing point is responsible for the higher critical temperature (15-19°), and factors other than lipid melting properties, perhaps cytoskeleton structure, contribute to the lower critical temperature (5-7°) for lectin interactions with the exposed surface of LM cells.

INTRODUCTION

A number of well characterized membrane proteins are integrated within the lipid bilayer (1-2). At least some of these "integral" membrane proteins can move laterally within the plane of the membrane (3-4). The concanavalin A (Con A) receptor protein has been shown to be induced into a more clustered arrangement in several transformed cell lines by the multivalent plant lectin Con A (1,5-6). Con A receptors have been demonstrated to be glycoproteins in several cell types (7-8) and transverse the entire lipid bilayer in the human erythrocyte membrane (9).

Lectin-induced clustering of Con A receptors may be a requirement for cell agglutination and would require fluid membrane lipids. Physical and physiological studies in Escherichia coli reveal characteristic temperatures that are responses to changes in the physical state of membrane lipids (10).

The higher characteristic temperature is that at which the formation of solid patches of membrane lipids is first detected. The lower characteristic temperature is the temperature at which essentially all membrane lipids are in a solid phase. Certain cytoplasmic membrane phenomena in eucaryotic cells also show temperature transitions as revealed by Arrhenius plots of amino acid transport (11) and electron spin resonance techniques (11-12).

The related phenomena of Con A binding and Con A-mediated agglutinability have recently been shown to decrease dramatically at 15° for 3T3 cells (13-14) and 15-19° for mouse LM cells (15). A second critical temperature at 5-7° was observed for these Con A interactions with LM cells (15).

In the present study we report the effects of changes in fatty acid composition in LM cells on the characteristic temperatures of Con A binding and Con A-mediated agglutinability.

METHODS

Mouse LM cells derived from NCTC clone 929 (L cells) were maintained on Eagle's minimal essential medium (MEM) with Earle's salts plus 0.5% Difco bacto-peptone (MEM + P) as described previously (16). The procedure used to alter the fatty acid composition of LM cell lipids by enriching for polyunsaturated or saturated fatty acids is essentially that described earlier (17). At confluency, cells were removed from tissue culture flasks with 0.005% trypsin (Sigma: type 2, pancreatin; 1500 BAEE units per mg) and plated at a 20-fold lower cell density on Linbro multi-dish trays (35 mm diameter). The cells were incubated for 24 hr in MEM modified to contain twice the normal glutamine and vitamin concentrations and 2 mg of the biotin antagonist, dl-desthiobiotin, per liter (MEM + GVdB). The medium was then replaced with MEM + GVdB containing a fatty acid covalently attached to a detergent (Tween). Tween-nonadecanoic acid (Tween-19:0) at 8 μg/ml of fatty acid equivalent (fae) was used to increase the proportion of saturated fatty acids in phospholipid and Tween-linolenic acid (Tween-18:3) at 8 μg/ml (fae)

was added to increase double bond content of fatty acids in phospholipid.

Con A (3X crystallized, Miles Laboratories) was labeled with [^{3}H]acetic acid anhydride (500 mCi/mmole, Amersham/Searle) by the method of Agrawal et al. (18).

RESULTS

The percentage of saturated fatty acid in phosphatidylcholine and phosphatidylethanolamine for cells grown with 8 μg/ml (fae) of Tween-19:0 or Tween-18:3 was approximately 45% and 30% respectively as reported previously (17). The unsaturated fatty acid content of phospholipids in control cells (propagated in MEM + P) was approximately the same as in cells grown with Tween-18:3. However, the degree of unsaturation is greater after enrichment with the polyunsaturated supplement Tween-18:3 since approximately 11% of the fatty acids were derived from linolenic acid. Control cells contain no detectable polyunsaturated fatty acids.

Cells enriched with nonadecanoic acid (higher saturated/unsaturated ratio) display the same lower characteristic temperature for Con A related phenomena as control cells. For cells enriched with nonadecanoic acid, a marked increase in Con A binding occured at 5-9° compared to 5-7° for control cells (MEM + P) (Fig. 1A and 1B). In contrast, however, the upper characteristic temperature increased approximately 7° for C_{19} enriched cells (22-28°), and binding of Con A to C_{19} enriched cells had not reached a plateau at 35°.

The lower critical temperature for Con A binding was not altered in $C_{18:3}$ enriched cells, whereas the upper critical temperature was shifted downwards (7-11°) compared to control cells (15-19°) (Fig. 1B and 1C).

At ice bath temperature, the saturating concentration for lectin binding was approximately the same both for control cells and for cells enriched for saturated or unsaturated fatty acids (not shown), indicating that fatty acid composition does not significantly alter the availability of Con A receptors.

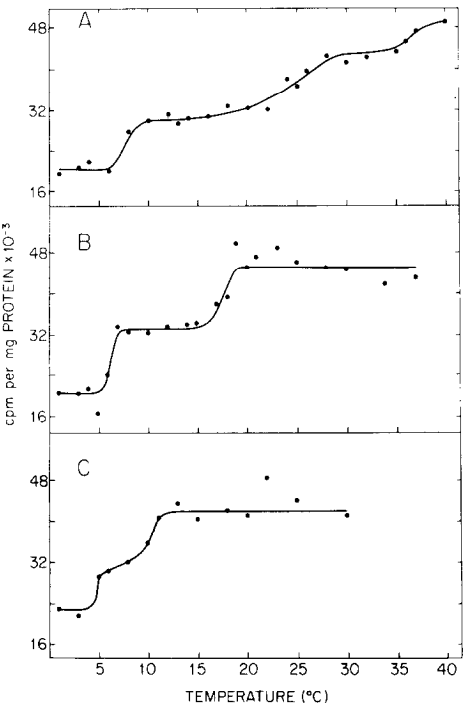

FIGURE 1. Binding of radioactively labeled Con A to LM cells. Cells were incubated 10 min at the test temperature, washed twice with 0.85% NaCl (saline) and incubated 5 min at the test temperature with [^{3}H]-Con A (100 µg/ml) in a 0.1 M Na_2HPO_4 buffer, pH 7.2 containing 0.85% NaCl and 0.001 M $MgCl_2$ (PBS). After five washes in saline the cells were suspended by incubation in 1 ml of 5% $NaCO_3$-0.1 N NaOH for 1 hr. The samples were divided into equal portions for measurements of tritium content in solution containing 3:1 toluene-Triton X-100 (19) and protein by the method of Lowry et al. (20). All points in Figs. 1-2 are the average of six determinations. (A), cells enriched with nonadecanoic acid ($C_{19:0}$). (B), control cells maintained on MEM + P. (C), cells enriched with linolenic acid ($C_{18:3}$).

The same general pattern of temperature shifts was observed for Con A-mediated hemadsorption to cells containing a higher or lower percentage of saturated fatty acids (Fig. 2). Cells enriched with nonadecanoic acid revealed an unaltered lower critical temperature (5-10°), but the upper critical temperature was elevated (22-26°) as compared with control cells (15-19°) (Fig. 2A and 2B). The lower characteristic temperature of Con A-mediated hemadsorption is more apparent at higher concentrations of Con A (100 and 200 µg/ml) as shown in Fig. 2B. Only a single characteristic temperature was observed for Con A-mediated hemadsorption to cells enriched

with linolenic acid. Apparently the upper critical temperature was lowered to 7-11° (Fig. 2C) and coincided with the lower characteristic temperature observed in control cells (Fig. 2B).

DISCUSSION

The critical temperature of Con A binding and hemadsorption to LM cells enriched with C_{19} and $C_{18:3}$ fatty acids occurred over a broad range compared with the behavior of these phenomena in control cells (Fig. 1 and Fig. 2). This may have resulted from the creation of heterogeneous populations of cells during growth with fatty acid supplements. Only a few doublings in

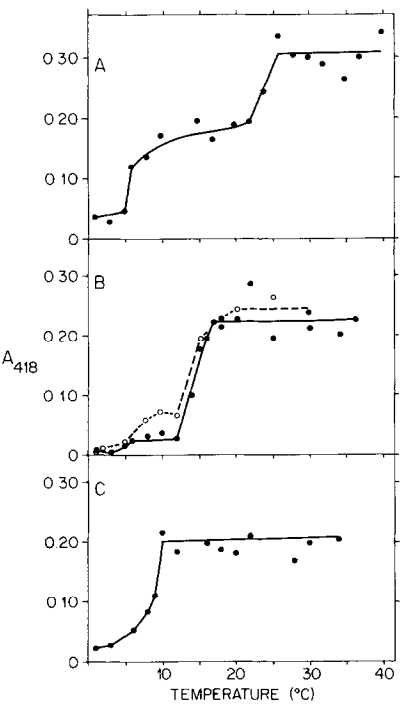

FIGURE 2. Con A-mediated hemadsorption to LM cells. Cells at 80-100% confluency in Linbro multi-dish wells were incubated for 10 min at the test temperature, washed twice with saline and incubated 5 min at the test temperature with Con A in PBS, pH 7.2. The cells were then washed five times with saline and incubated for 10 min with rabbit erythrocytes (2% v/v) in PBS. Finally, the cells were washed five times with PBS, solubilized in 5% sodium dodecyl sulfate (w/v) and analyzed spectrophotometrically for hemoglobin content at 418 nm. All washings were done at the test temperature. (A), cells enriched with $C_{19:0}$, 200 µg/ml Con A. (B), control cells maintained with MEM + P, ○—○, 200 µg/ml Con A; ●—●, 100 µg/ml Con A. (C), cells enriched for $C_{18:3}$, 100 µg/ml Con A.

cell number occurred during the fatty acid enrichment procedure outlined here. This could give rise to populations of cells which differ in fatty acid composition.

The shift toward a higher or lower upper characteristic temperature for Con A binding or Con A-mediated hemadsorption for cells with a higher or lower degree of saturation in membrane lipid fatty acids respectively is that predicted if these phenomena are responsive to phase changes in membrane lipids (10,21-23). As the degree of saturation decreases or increases the freezing point of membrane lipids would be expected to decrease or increase respectively. Recent studies in which we have employed cytoplasmic membranes from LM cells and electron spin resonance probes to detect the beginning and end of the phase transition indicate that the upper characteristic temperature for the Con A related phenomena reported here correlates with the lower characteristic temperature of the membrane lipid phase transition, i.e. the end point for freezing of the membrane lipids (11). The critical temperatures for Con A-mediated hemadsorption cannot be a consequence of decreases in Con A binding at lower temperatures (see Fig. 1 and 2). The same critical temperatures for hemadsorption were observed in an experiment where Con A binding to cells was performed at 22° for all samples and the excess Con A was removed prior to assaying hemadsorption at different temperatures (24). No significant loss of labeled Con A bound at 22° occurred after shifting to lower or higher temperatures (24).

The finding that the lower characteristic temperature for Con A binding and Con A-mediated hemadsorption for control cells (5-7°) was essentially unchanged for cells enriched for either saturated or polyunsaturated fatty acids corroborates previous data which suggested that this lower critical temperature arises from factors other than membrane lipid fluidity (15).

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