A COMPARISON OF LIPID LATERAL DIFFUSION IN THE CELLULAR PLASMA MEMBRANE AND IN MULTIBILAYERS COMPOSED OF PLASMA MEMBRANE LIPIDS

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In this experiment, the diffusion of dihexadecylindocarbocyanine (diI-C_{16}[3]), a fluorescent lipid analogue, was compared in several systems: the plasma membrane (PM) of living human fibroblasts; the substrate-attached surface membrane remaining after the top surface, nucleus and cytoplasm were removed; multibilayers reconstituted from the whole cell lipids (WCL); and multibilayers reconstituted from plasma membrane lipids (PML). Full details of this study will have been reported.[1] One major purpose was to inquire about the effects of membrane proteins on the lateral mobility of lipids in the plasma membrane. The human fibroblasts chosen for this project were highly spread and only slowly internalized the lipid analogue probe. Large numbers of these cells were fractionated on a sucrose step gradient to obtain plasma membranes (Kartner et al., 1977). In our PM preparation 5′ nucleotidase, a PM marker, was enriched 10-fold while succinate dehydrogenase, a mitochondrial marker, and nucleic acids were depleted by 20- and > 10-fold, respectively. Lipids from this fraction and the entire cell were extracted and analyzed as to lipid class and acyl chain composition. Cell surface (Jacobson et al., 1977) and multibilayer (Wu et al., 1977) diffusion of diI-C_{16}(3) were measured by the technique of fluorescence recovery after photobleaching (FRAP).

[1]Jacobson, K., Y. Hou, Z. Derzko, J. Wojcieszyn, and D. Organisciak. 1981. Lipid lateral diffusion in the surface membrane of cells and in multi-bilayers formed from plasma membrane lipids. *Biochemistry*, 20:5268.

RESULTS AND DISCUSSION

Diffusion coefficients (D) for diI-C_{16}(3) inserted into the living cell's plasma membrane or the substrate-attached membrane alone were similar, ranging from $\sim 3.5 \times 10^{-9}$ cm^2/s at 5°C to $\sim 2 \times 10^{-8}$ cm^2/s at 37°C with a gradual change in slope occurring in the neighborhood of 25°C (bottom curve, Fig. 1). For diI-C_{16}(3) incorporated into extracted plasma membrane lipid multibilayers, D ranged from $\sim 5 \times 10^{-9}$ cm^2/s at 5°C to over 6×10^{-8} cm^2/s at 37°C (middle curve, Fig. 1). When the probe was introduced into multibilayers formed from the whole cell lipids, D was $\sim 9 \times 10^{-9}$ cm^2/s at 5°C and increased to 8.5×10^{-8} cm^2/s at 37°C (top curve, Fig. 1). The diffusion coefficient-temperature curves for both of the multibilayers displayed a discontinuity at ~ 10°C and a change in slope near 25°. (The diphenylhexatriene (DPH) emission anisotropy vs. temperature curve for WCL multilamellar vesicles showed a slight sigmoidicity, with the region of steepest slope occurring between 11°C and 28°. These temperatures approximately correspond to the discontinuity and slope change, respectively, in the WCL multibilayer lateral diffusion data.) Above 25°C, the temperature dependence of D was similar in all systems with activation energies (E_a) in the range of 5–7 kcal/mol, which is typical for fluid phase diffusion in simple artifical bilayers. Below 25°, E_a ranged from 9–11 kcal/mol for the intact cells and bottom surface ghosts to 17 kcal/mol for the PM lipid multilayer.

In comparing diffusion in the PML and WCL multibilayers, the major compositional difference is the increased

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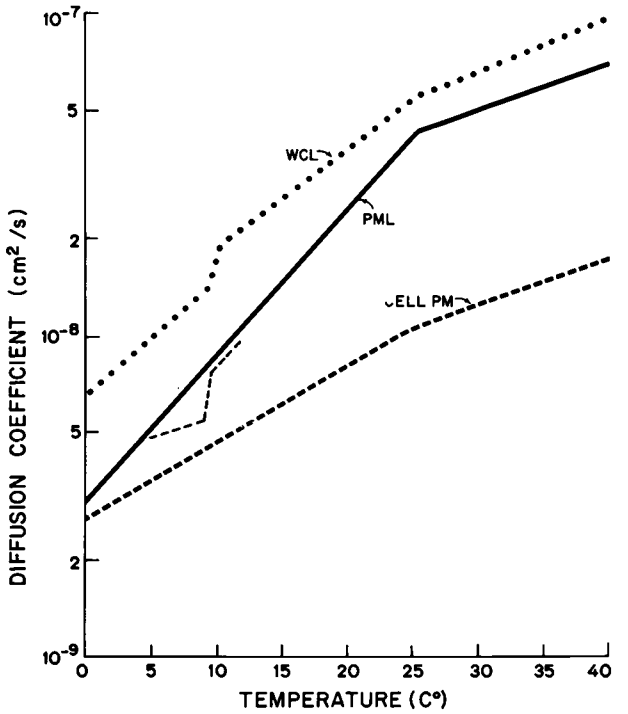

FIGURE 1 Top curve (•), dil-C_{16}(3) diffusion in multibilayers formed from the whole cell lipid (WCL) extract. Middle curve (⁻), dil-C_{16}(3) diffusion data in multibilayers formed from the extracted plasma membrane (PML) lipids. In this case, data from four independent experiments were collected from temperature intervals ≤ 2.5°C and averaged; the dotted segment near 10°C represents a feature seen in several individual experiments but which disappeared in the selected data averaging process. Bottom curve (--), dil-C_{16}(3) diffusion coefficients as obtained from FRAP experiments on living human fibroblasts; the cells were stained with ~ 2 μg/ml of the fluorescent probe following its injection into the bathing solution from a concentrated ethanol solution. (Full details have been reported by Jacobson et al.[1])

cholesterol to phospholipid ratio in the PM lipids, with the other differences being minor. This difference can account for the decreased PML bilayer fluidity above 25°C. The sudden decrease in diffusion rate at ~ 10°C could be explained by a generalized transition decreasing *D* by a factor or two. However, since the DPH anisotropy data show only a slight change in slope in this region, a bulk transition seems extremely unlikely. Rather, as the sample is cooled below 25°C, a lateral phase separation may ensue with the probe distributing in a characteristic fashion among the various phases present. Such a separation is suggested by the higher diffusion activation energies below 25°C. In this view, the segregation of the probe reaches an abrupt completion at 10°C, at which point it is totally localized in a lower fluidity phase.

The difference in diffusion rates for dil-C_{16}(3) between the PM and PML multilayer at the higher temperatures presumably is due to the presence of (glyco-) proteins in the PM. Membrane proteins could decrease bilayer fluidity generally or specifically bind the dil-C_{16}(3) probe. These effects could reduce the lateral diffusion rates in the PM relative to a bilayer composed of its lipids. The overall closeness of the results for lipid analogue lateral diffusion in the plasma membrane and in simpler bilayers constructed from the PM lipids suggests that the Fluid Mosaic model (Singer and Nicolson, 1972) provides a correct qualitative description of lipid lateral mobility. Relatively minor modification based on current thinking about protein-lipid interactions should account for the observed differences. However, these results are in marked contrast to those emerging from a comparison of the lateral diffusion of (glyco-) proteins in cell membranes and in reconstituted bilayers. Such results indicate that protein diffusion in bilayers is at least an order of magnitude faster than in cell surface membranes (see Jacobson and Wojceiszyn, 1981, and references therein). Thus, to explain protein mobility in cell surface membranes, more extensive modification of the original fluid mosaic model is required, presumably involving structures peripheral to the membrane (Jacobson and Wojcieszyn, 1981).

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