

Reduced lateral mobility of a fluorescent lipid analog in cell membranes of rat fibroblasts transformed by simian virus 40

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Received 13 January 1989

In order to study the difference between normal and transformed cells, lateral motion of fluorescent molecules embedded into cell membranes of rat clonal fibroblasts and its SV40-transformed derivative cells was measured by the FPR technique. The lateral diffusion coefficient of a fluorescent fatty acid analog, F18, was smaller in transformed cells than normal cells. This indicates that the lipid phase of membranes from transformed cells is less fluid than that from normal cells. On the other hand, the lateral diffusion coefficient of S-F-concanavalin A was identical in both cells. These results suggest that the mobility of different molecules on the membranes is controlled by different mechanisms.

Virus-induced transformation; Fluorescence photobleaching recovery; Lateral motion; (Fibroblast)

1. INTRODUCTION

Transformation of cells by virus induces many changes such as altered morphology and cytoskeleton, reduced fibronectin synthesis, altered structure of glycolipids and glycoproteins, and release from contact inhibition. In the mechanisms of these phenomena, it is plausible that the dynamic properties of cell membranes such as membrane fluidity and mobility of molecules may

play important roles. Take contact inhibition for example, fluid membranes are energetically stable for close contact. Indeed, the mobility of lipophilic fluorescent molecules in the membranes of virus-transformed fibroblasts is lower than that of normal cells [1,2]. On the other hand, the mobility of concanavalin A bound to surface membranes of fibroblasts is higher in transformed cells [3]. However, most of these data are based on the measurements of rotational mobility. Rotational mobility is composed of the over-all rotation of molecules as well as side chain rotation. Also, wobbling, twisting and bending motion influence the measurement of rotational mobility. On the other hand, lateral mobility is composed of only over-all motion. In order to obtain more clear information on the dynamic properties of cell membranes, combination of the data of rotational and lateral mobility is required. The lateral mobility of F18 and S-F-concanavalin A is very sensitive to the structural changes of cell membranes from neuronal cells [4-6] and muscle cells [7]. Here we report that the lateral mobility of fluorescent molecules on cell membranes measured by the FPR technique is different depending on probes used: the lateral mobility of fluorescent fatty acid analog

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Abbreviations: S-F-concanavalin A, succinyl concanavalin A conjugated with fluorescein isothiocyanate; F-concanavalin A, concanavalin A conjugated with fluorescein isothiocyanate; F18, 5-(octadecylthiocarbamoylamino)fluorescein; SV40, simian virus 40; FPR, fluorescence photobleaching recovery

was lower in transformed fibroblasts, whereas, the lateral mobility of S-F-concanavalin A remained unchanged.

2. MATERIALS AND METHODS

2.1. Cell lines and cell culture

The 3Y1-B clone 1-6, a cell line of Fisher rat embryonic fibroblasts, and a derivative line W-3Y-23, transformed by SV40, were kindly provided by Dr Yamaguchi, Institute of Medical Science, University of Tokyo [8]. The cells were grown on coverslips in plastic dishes in Eagle's minimum essential medium containing 10% new born calf serum and antibiotics (100 U penicillin, 100 µg streptomycin and 60 µg kanamycin per ml of medium). The culture was performed in a humidified atmosphere of 95% air and 5% CO₂ at 37°C.

2.2. Fluorescent probes

A fluorescent analog of fatty acid, F18, was synthesized as in [9]. The preparation of S-F-concanavalin A was described in [7].

2.3. Labelling of cells

Cells grown on coverslips for 2 days were washed twice with Hanks' buffer. The washed cells were incubated in Hanks' buffer containing 10 µM F18 for 2 min at 37°C or in Hanks' buffer containing 200 µg/ml of S-F-concanavalin A for 15 min at 37°C. Then the coverslips were washed 4 times with Hanks' buffer. They were set in a temperature-controlled quartz container and were placed on the stage of a microscope.

2.4. Measurements

The lateral mobility of fluorescent molecules was measured by FPR. Instrumental description and analysis are given in [4]. The diameter of the bleached area was 3.8 µm using a 100× objective lens and a pinhole. In order to reduce the effect of rotational motion, a polarizer and a 1/4 wavelength phase plate were inserted along the path of exciting light as described in [4]. Only the uniformly labelled regions of cell surfaces were selectively measured in order to reduce the effect of aggregation and internalization of the probes. Recovery fraction, *f*, and lateral diffusion coefficient, *D*, were determined using following equations:

$$f = (I_{\text{inf}} - I_{\text{bl}}) / (I_{\text{ini}} - I_{\text{bl}})$$

$$D = 0.22\omega^2 / t_{1/2}$$

*I*_{ini}, *I*_{bl} and *I*_{inf} are fluorescence intensities before bleaching, just after bleaching and a long time after bleaching when recovery is saturated, respectively; ω is the radius of bleached area and *t*_{1/2} is the recovery half-time.

3. RESULTS

Table 1 shows the lateral diffusion coefficients of F18 embedded into cell membranes of normal and SV40-transformed fibroblasts. These values were close to those of membrane lipids [10,11] and were almost identical to those obtained in other

Table 1

Lateral diffusion coefficient and recovery fraction of F18 labelled rat clonal fibroblasts, 3Y1-B, and SV40-transformed cells, W-3Y-23

	<i>D</i> ₂₅ (cm ² /s)	<i>f</i>	Number of cells measured
3Y1-B (normal)	$(2.1 \pm 0.3) \times 10^{-9}$	0.98 ± 0.03	9
W-3Y-23 (transformed)	$(1.4 \pm 0.4) \times 10^{-9}$	0.96 ± 0.05	14

*D*₂₅ is the lateral diffusion coefficient at 25°C. All measurements were completed within 40 min after labelling. The difference in *D*₂₅ is significant by *P* < 0.01

cells [4-7]. Therefore, the motion of F18 may represent that of membrane lipids and the membrane fluidity, as described by the lateral mobility of F18, was similar in various cell types. It is clear that the lateral diffusion coefficient of F18 was smaller in transformed cells. On the other hand, recovery fraction, *f*, was almost 100% with no difference between normal and SV40-transformed cells. This means that all F18 molecules are mobile and only their lateral mobility is reduced in transformed cells.

Table 2 shows the lateral diffusion coefficients of S-F-concanavalin A labelled cell membranes of normal and SV40-transformed cells. According to table 2, the mobility was about 10 times less than that of F18. This suggests that the motion of S-F-concanavalin A represents that of membrane proteins rather than membrane lipids. In contrast to F18, the lateral mobility of S-F-concanavalin A showed no difference between normal and transformed cells. It should be noted that despite the

Table 2

Lateral diffusion coefficient and recovery fraction of S-F-concanavalin A labelled rat clonal fibroblasts, 3Y1-B, and SV40-transformed cells, W-3Y-23

	<i>D</i> ₂₅ (cm ² /s)	<i>f</i>	Number of cells measured
3Y1-B (normal)	$(1.3 \pm 0.4) \times 10^{-10}$	0.50 ± 0.11	10
W-3Y-23 (transformed)	$(1.2 \pm 0.6) \times 10^{-10}$	0.52 ± 0.12	11

*D*₂₅ is the lateral diffusion coefficient at 25°C. All measurements were completed within 40 min after labelling

fact that visible patches on the cell surface were avoided in measurements, only half of the S-F-concanavalin A was mobile.

4. DISCUSSION

Here, the lateral mobility of fluorescent probes embedded in cell membranes of normal and SV40-transformed fibroblasts has been compared. If the probes are freely moving in the membrane, its mobility is determined by the fluidity around the probes, the volume of the probes and temperature. In this case, both lateral and rotational measurements give similar tendencies. According to table 1, the lateral diffusion coefficient of F18 was smaller in transformed cells than in normal cells. Previous studies showed that the rotational mobility of lipophilic fluorescent probes was reduced in transformed fibroblasts [1,2]. Since both the rotational and lateral mobility of lipophilic probes were reduced in transformed fibroblasts, it can be concluded that the lipid phase of cell membranes from transformed fibroblasts has a lower fluidity.

What is responsible for this lower membrane fluidity? In neuroblastoma cells [12] and in lymphocytes [13], parallel correlation between reduced mobility of fluorescent probes and increased cholesterol content was reported. It is known that SV40-transformed fibroblasts contain more cholesterol than normal cells [2], therefore, the increased cholesterol content may be one reason for reduced membrane fluidity in transformed fibroblasts.

The lateral motion of S-F-concanavalin A did not differ between normal and transformed fibroblasts in the present experiment. On the other hand, Inbar [3] reported that rotational motion of F-concanavalin A was faster in SV40-transformed fibroblasts. The apparent inconsistency from our result can be explained as follows. (i) For the measurements of rotational motion of F-concanavalin A, the fluorescence anisotropy technique was used. Due to the short fluorescence life time of fluorescein (less than 5 ns), only side chain motion of F-concanavalin A was measured in their experiments. For translational motion, however, the entire surface of S-F-concanavalin A is involved. Therefore, friction in a limited region around concanavalin A may be reduced in transformed fibroblasts, and this reduction will not influence greatly the over-all motion of concanavalin

A. (ii) It is known that cell structure is dependent on whether cells are attached or not to the substrate. In Inbar's experiment, the cells were dissociated from substrate by EDTA or trypsin, while in the present experiment, the measurements were performed in attached state. This difference may be reflected in the motion of S-F-concanavalin A. (iii) Both concanavalin A and succinyl concanavalin A are easily aggregated when they are bound to cell membranes. In the present experiment, visible aggregates of S-F-concanavalin A were observed at cell surfaces and around nuclei. These large aggregated regions were avoided and uniformly labelled regions were selectively measured. In Inbar's experiment, however, this discrimination was not performed. Therefore, not only the side chain motion of freely mobile F-concanavalin A, but also the motion within the aggregated region contributed to the averaged rotational mobility. The mobility and/or the relative fluorescence intensity in the aggregated region may change upon transformation. This may be the third reason.

Nicolson [14] reported that the lateral mobility of F-concanavalin A is increased on SV40-transformed fibroblast cell membranes. His conclusion is also inconsistent with the present result. He measured the lateral mobility by observing patch formation. This method is indirect and the results are not necessarily explained only by lateral mobility. The concentration of concanavalin A receptors and the ability of concanavalin A and receptor complexes to aggregate also contribute to patch formation. In our cell lines, a significant difference in patch formation between normal and transformed cells was not observed.

In the present experiment, lateral motion of F18 showed the reduced mobility in transformed cells. On the other hand, that of S-F-concanavalin A showed no difference. How can these results be explained? Unfortunately, we do not have any clear reasons at this stage. However, the following can be speculated. According to Koppel et al. [15], lateral motion of membrane proteins is controlled by matrix structures just beneath cell membranes (may be membrane undercoat or cytoskeletal structures). If this is true, fluidity of membranes may decrease upon transformation but not the fluidity of the matrix structures.

The cells, which exhibit contact inhibition, are

usually tightly adhered to one another. In this case, fluid membranes may be energetically more favourable than the hard membrane. This may be one of the biological reasons why normal fibroblasts have more fluid membranes than transformed ones. Indeed, in myogenic cells, the mobility of the molecules in the cell membranes increased prior to cell fusion when the cells were in close contact [7].

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