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EVIDENCE OF FAR ULTRAVIOLET LIGHT-MEDIATED CHANGES IN PLASMA MEMBRANE STRUCTURE AND FUNCTION

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Irradiation of plasma membrane preparations with 254 nm light increases its apparent microviscosity as measured with fluorescent polarimetry. Doses of $3 \cdot 10^4$ J/m^2 increase the fluorescent polarization of a diphenylhexatriene probe by 10%. A similar increase is seen when whole cells are irradiated. The fate of membrane protein following irradiation was examined using SDS-polyacrylamide gel electrophoresis. Increasing the 254 nm doses reduces the amount of material in distinct bands on the gel and increases the amount of very low mobility material. No new bands of Coomassie blue staining material were observed. Irradiation of whole cells inhibited their attachment to concanavalin A-coated surfaces, an indication of a change in membrane function.

Introduction

Far ultraviolet irradiation is sometimes used to sterilize materials for tissue culture investigations. For example, membrane preparations sterilized with about 10000 J/m^2 have been found to inhibit 3T3 cell division by about 40% [1]. These results have been interpreted to indicate that normal membrane to membrane interactions are involved in contact inhibition. This conclusion does not consider the possibility that the sterilization procedure alters physical and chemical properties of the plasma membrane. For example, irradiation of erythrocytes with an ultraviolet light dose of 6000 J/m^2 leads to increased membrane permeability to sodium and potassium ions and in turn leads to hemolysis [2]. A possible mechanism for this effect is the photoperoxidation of membrane lipids. Evidence of photoperoxidation has been obtained at doses of less than 5000 J/m^2 [3].

In this note we demonstrate that sterilizing doses of ultraviolet light alter physical, chemical and functional properties of the plasma membrane. These alterations in fundamental properties of membranes should be considered when interpreting data from experiments using ultraviolet-sterilized membrane preparations.

Materials and Methods

Derivatization of disks. Disks of polyethylene were cut from sheets with a 1.25 cm diameter die. The disks were derivatized with concanavalin A (Sigma) by floating the disks on a solution of 100 μg/ml concanavalin A and 2.5% glutaraldehyde. After 30 min the disks were inverted and incubation continued, with light oscillation, overnight. The disks were then washed in buffer and stored in 0.3 M glycine to inactivate residual glutaraldehyde. Just before use the disks were washed in phosphate-buffered saline.

Tumor cells. Ascitic mouse ovarian tumor cells [4] were grown in C3G/HeJ mice (Jackson Laboratory, Bar Harbor, ME). The night before use the mice were injected with 0.2 mCi tritiated thymidine (New England Nuclear, Boston, MA) intraperitoneally. The fol-

Abbreviation: SDS, sodium dodecyl sulfate.

lowing morning the cells were removed, washed three times in phosphate-buffered saline and resuspended in phosphate-buffered saline to a concentration of about 10^8/ml.

Attachment assay. 1 ml of cells at 10^7/ml were poured into a 35-mm plastic petri dish, three disks placed into the suspension and incubated at room temperature. After 10 min the dish was shaken to resuspend the cells and incubation continued for an additional 10 min. The disks were then washed in phosphate-buffered saline, and counted in Aquasol (New England Nuclear) in a Beckman liquid scintillation counter.

Membrane preparation. Membranes from mouse ovarian tumor cells were prepared as described by Brunette and Till [5] using a dextran/polyethylene glycol two-phase system.

Fluorescent polarization measurements. Using a diphenylhexatriene probe (Sigma Chemical Co., St. Louis, MO) whole cell and isolated plasma membrane steady-state fluorescent anisotropy was measured as described by Shinitzky and Inbar [6] using an Elscint polarimeter (Elscint Ltd., Haifa, Israel). Since diphenylhexatriene absorbs ultraviolet light we were concerned that the presence of this fluor might affect the response of membrane preparations to irradiation. Thus, diphenylhexatriene was added either before or after irradiation. Fig. 2 shows that the results were quantitatively similar in both cases.

Irradiation. Irradiation was at 12 cm from two Sylvania G8T5 bulbs with slow stirring. The incident ultraviolet light (254 nm) dose rate was measured with a Black-ray ultraviolet meter J-225 (Ultraviolet Products Inc., San Gabriel, CA) and found to be 12 J/m^2 per s.

Polyacrylamide gel electrophoresis. Membrane preparations were suspended at 80 μg (protein)/ml in 1 ml water and irradiated. The suspension collected, centrifuged at 1 600 X g and the pellet applied to a discontinuous SDS slab gel prepared following the procedures of Laemmli [7]. Proteins were stained with Coomassie blue.

Results and Discussion

Rapid attachment of tumor cells to lectin-coated plastic surfaces is an indication of normal membrane function. Although attachment is mediated by the binding of cell surface carbohydrates to lectin receptors it is not merely a passive binding reaction. Metabolic energy [8] and a functional cytoskeleton (Braun, A.G. and Emerson, D.J., unpublished data) are required for attachment. In addition, attachment is thought to be mediated by short-range lateral motion of cell surface structures [8].

Thus the attachment of tumor cells to concanavalin A-coated plastic surfaces may be a useful test for the functional integrity of the plasma membrane. Since we were concerned that ultraviolet light might alter membrane structure we have used attachment as a preliminary test for membrane function following irradiation. Fig. 1 shows that attachment is inhibited by increasing doses of ultraviolet light. Thus, at least this membrane-mediated function is disrupted by sterilizing doses of ultraviolet light.

Concanavalin A-mediated agglutination of tumor cells is not inhibited by irradiation. Indeed, the data shown in Table I indicate that irradiated cells were more readily agglutinated by concanavalin A than were unirradiated or sham-irradiated cells. While the mechanism of this enhanced sensitivity is unclear

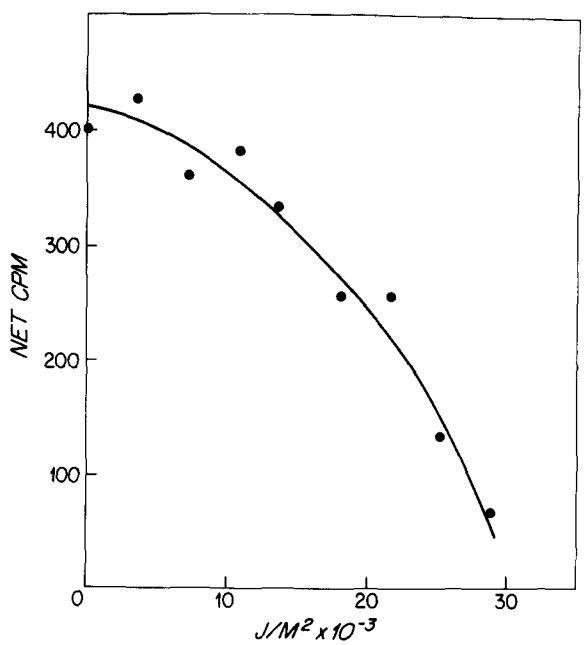

Fig. 1. Effect of 254 nm ultraviolet light on tumor cell attachment to concanavalin A-coated disks. Cell suspensions were irradiated and immediately tested for attachment as described in Materials and Methods.

TABLE I

EFFECT OF IRRADIATION ON AGGLUTINATION

Mouse ovarian tumor cells at $8 \cdot 10^5$ in phosphate-buffered saline were incubated with indicated concentration of concanavalin A at room temperature for 10 min and judged for agglutination according to this code: –, no agglutinated cells; ±, less than 1/3 of cells clumped; +, between 1/3 and 2/3 cells clumped; ++, more than 90% of cells clumped; +++, all cells in very large clumps (more than 20 cells/clump). Cells were irradiated with slow stirring, Sham-irradiated cells were stirred but not irradiated for the same time interval.

Treatment	Concanavalin A (μg/ml)				
	0	50	75	100	125
None	–	±	+	++	++
Sham-irradiated	–	±	+	++	++
30 000 J/m^2	–	+	+++	+++	+++

these data imply that irradiation does not reduce materially the number of exposed concanavalin A-binding sites.

Since lipid peroxidation has been noted following ultraviolet light irradiation of artificial and natural membranes [9] we attempted to prevent ultraviolet-light-mediated attachment inhibition with catalase. Concentrations of 15 300 units/ml did not interfere with the ultraviolet light effect.

Agents that crosslink cell surface elements, such as glutaraldehyde, inhibit attachment [10]. Thus, one possible mechanism for the inhibition of attachment is through a ultraviolet light-mediated crosslinking of cell surface elements. It seemed possible that such crosslinking may be reflected in a reduction in the mobility of cell surface elements. This possibility was tested using a well known polarization probe, diphenylhexatriene, to determine the effect of ultraviolet irradiation on probe mobility. As seen in Fig. 2, ultraviolet light causes an increase in polarization in a dose-dependent manner, a result that suggests an increase in cell surface microviscosity [6] or an increase in the structural order of the membrane [11]. Recently, however, it has been suggested that polarization measurements of whole cells using the diphenylhexatriene probe are not an accurate measure of plasma membrane polarization since the probe can enter the cytoplasm of some cells and interact with internal structures [12]. A less ambiguous measure of plasma changes following ultraviolet light irradiation was achieved by isolating the plasma membrane, irradiating samples, adding the probe and measuring fluorescent polarization. The result of such an experiment are also shown in Fig. 2. Here a linear relationship between polarization and ultraviolet light dose is seen, again suggesting that ultraviolet light alters the organization of the plasma membrane. The non-linear whole cell response of polarization as a function of ultraviolet light dose may be due to the diphenylhexatriene probe entering relatively ultraviolet light-resistant cytoplasmic structures.

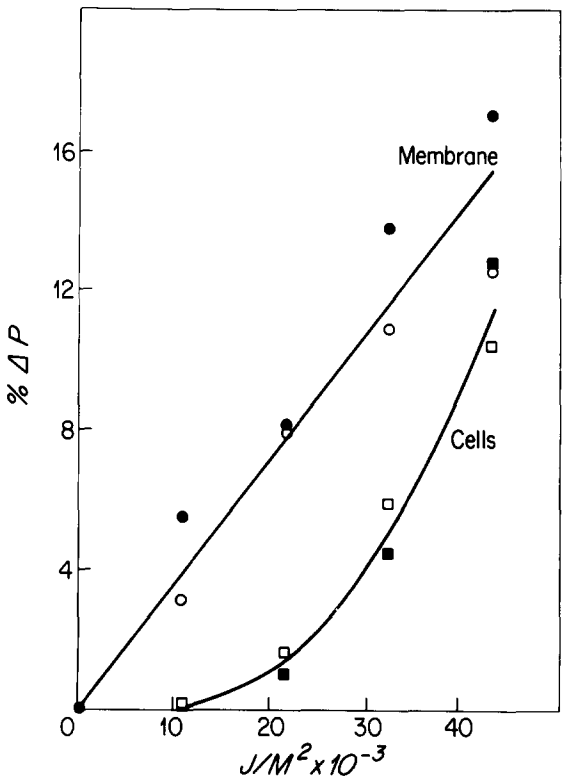

Fig. 2. Effect of pre-treatment with 254 nm ultraviolet light on diphenylhexatriene fluorescent polarization in cells and membranes. Two types of experiment are shown. In the lower curve the effects of ultraviolet light on whole cells was determined. Symbols: □, probe incorporated into cells before ultraviolet light irradiation; ■, probe incorporated into cells after irradiation. The methods were otherwise identical with the addition of diphenylhexatriene following ultraviolet light irradiation. Initial polarization, 0.203. In the upper curve the data from two independent isolations of cell membranes are shown: ○, experiment 1, initial polarization, 0.229; ●, experiment 2, initial polarization, 0.220.

If ultraviolet light causes crosslinking of plasma membrane elements it should be possible to demonstrate this change directly with gel electrophoresis. Fig. 3 shows the electrophoretic pattern of membrane proteins at several ultraviolet light doses. Identical quantities of membrane were applied to each lane of the gel. As the ultraviolet light dose increases the bands in the gel disappear and are replaced by increasing amounts of Coomassie blue staining material at the top of the gel. Indeed, much of this new material does not even penetrate the stacking gel, an indication of very high molecular weight material. No new bands appear following irradiation. Slight changes in the electrophoretic pattern of irradiated membrane preparations are visible at doses of 2 700 J/cm^2 on the original gel.

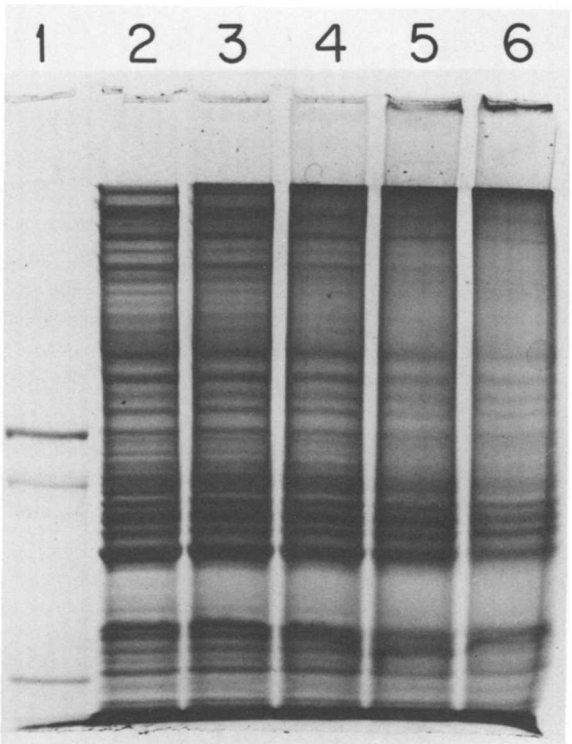

Fig. 3. SDS-polyacrylamide gel electrophoresis of membrane following ultraviolet light irradiation. Isolated plasma membrane vesicles were prepared as described by Brunette and Till [5], irradiated, subjected to SDS-polyacrylamide gel electrophoresis as described by Laemmli [7] and stained with Coomassie blue. The stacking gel was not removed. Lane 1, standards, bovine serum albumin (66 000 daltons), catalase (57 000 daltons) and concanavalin A monomer (25 500 daltons); lane 2, unirradiated membrane; lane 3, irradiated with 2 700 J/m^2; lane 4, 5 400 J/m^2; lane 5, 10 800 J/m^2; lane 6, 21 600 J/m^2.

Thus, physical, chemical and functional changes occur in the plasma membrane following sterilizing doses of ultraviolet light. The interpretation of data using ultraviolet light-sterilized membranes should take these changes into account.

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