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Photoaffinity labeling of whole cells by flashed light: a simple apparatus for high-energy ultraviolet flashes

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A simple apparatus for the photolysis of affinity labels is described. A commercial quartz tube produces high-energy flashes (wavelength from 200 to 1000 nm). A single flash is normally sufficient to activate photoaffinity labels in the presence of cells. Flash photolysis has several advantages over continuous irradiation, e.g. there is no need for cooling and photolabeling may be performed after different preincubation periods. The above apparatus is therefore suitable for investigations on time-dependent uptake of substrates by intact cells. Examples are demonstrated by photoaffinity labeling of rat liver cells by [^{3}H]cyclosporin-diaziridine.

Introduction

Photoaffinity labeling of proteins is usually initiated by irradiation with ultraviolet lamps for several minutes [1,2]. Due to problems of cooling and sedimentation, this method is not suitable for labeling of membrane suspensions or cells. Long irradiation times may induce injury or structural changes and in addition, reactive groups may be exposed which are normally hidden.The usual procedure with ultraviolet lamps integrates a series of events that occur during the period of irradiation (10–20 min), whereas if the energy could be applied in a very short period all reactive groups couple with the label under identical conditions. Furthermore, the use of short flashes of light would allow a series of experiments with varied preincubation periods. Time-dependent distribution and permeation of photoaffinity labels can be studied in this manner. The only problem is the production of single flashes of high enough energy to photolyse adequate amounts of the label.

We here describe a simple and inexpensive apparatus for the production of high-energy flashes. Adequate caution is required for the safety of the experimenter; eye exposure must be prevented during the experiments.

Materials

The electronic equipment corresponds to similar constructions of commercial flash units for photography (Fig. 1). In a prototype we used

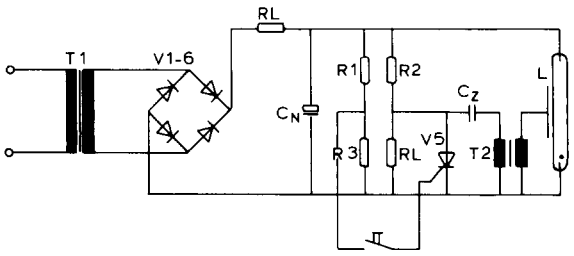

Fig. 1. The electronic equipment. $R_1 = 680\ k\Omega$, $R_2 = 330\ k\Omega$, $R_3 = 1\ k\Omega$, $R_4 = 300\ k\Omega$, $R_L = 2.4\ k\Omega$. $C_Z = 0.22\ \mu F$. $C_N = 2 \cdot A_N / U_N^2 \cdot 10^{-6}\ \mu F$ (A_N in W·s, U_N in V). V_1, ... V_4 = DSA 1.2–16A, V_5 = TIC 106. T_1 = sec. 450 V/0.5 A. T_2 = ZS 1052, L = EG 9902 (from Heimann GmbH, Wiesbaden-Dotzheim).

electrolytic capacitors from Siemens (1600 MF, 360 V + 20–10%). The quartz tube was purchased from Heimann GmbH, Wiesbaden-Dotzheim, (Type EG 9902, 500 V). Charging of the capacitors takes about 1 min. In our experience a single flash is sufficient for most procedures; therefore we did not modify the charging procedure. For C_N (Fig. 1) a capacitance of 4800 μF (630 V, 952 W · s) was installed in the prototype, but capacitances up to 8000 μF are useable. The quartz tube is contained in a light-tight box. Small amounts of cell suspensions can be exposed in an open petri dish or quartz tube fixed 2 cm below the flash tube. Volumes up to 30 ml can be treated, by placing a spiral quartz tube around the flash tube (Fig. 2).

In the experiments described here, filtering of the flashed light was not needed, but in some special cases optical filters are useful to prevent damage to cell membranes. Most of our experiments were carried out with freshly prepared rat hepatocytes (isolation according to Berry and Friend) [3] or plasma membranes prepared from rat liver according to Touster et al. [4]. We have had experience with the following photoaffinity labels in our laboratory: [^{3}H]cyclosporin-diaziridine (synthesized by Dr. R. Wenger, Sandoz AG, Basel, Switzerland); azido[^{14}C]benzamidotaurocholate (synthesized by Professor Dr. H. Fasold); [^{3}H]azido vasopressin (synthesized by Dr. F. Fahrenholz); [^{3}H]aflatoxin (purchased from Amersham Buchler GmbH, Braunschweig, F.R.G.). Cells or membranes in Tyrode buffer or phosphate-buffered saline (pH 7.4, 25°C) were exposed to flash-light. The samples were preincubated with the label in the dark for different time periods or temperatures. Photolysis was induced by a single flash of 1 ms at a distance of 2 cm from the flash tube. Cell or plasma membrane suspensions were exposed in quartz tubes of varying volumes. Pyrex or Duran tubes can be used, especially to cut off wavelengths below 300 nm. After photolysis, cells or membranes were extensively washed to remove unbound label. Protein content was evaluated by the method of Lowry et al. [5] using bovine serum albumin as a standard. Radioactively labeled proteins were identified by fluorography after SDS-polyacrylamide slab-gel electrophoresis [6].

Results

A precondition for any photoaffinity labeling procedure is a sufficient degree of photolysis. The results of a yield test are shown in Fig. 3. The photolytic degradation of [^{3}H]cyclosporin-diaziridine was 70% after one only flash, sufficient for a labeling procedure. The intensity of the flashed light can be modified either by variation of the distance between the quartz tubes or by modification of the capacitance. When the absorption maximum of the label is known, it is possible to use optical filters, but for most applications such care is not necessary.

In certain experiments, cell functions (such as transport, or cell viability) must be tested before and after photoaffinity labeling. For control purposes one flash was applied in the absence of photoaffinity label. In most preparations of liver cells, transport functions (or membrane permeability) were not drastically impaired by a single flash (Table I). In our laboratory, photoaffinity labels were activated by the apparatus described (Table II) using rat hepatocytes or isolated plasma membranes as targets. In particular, the method

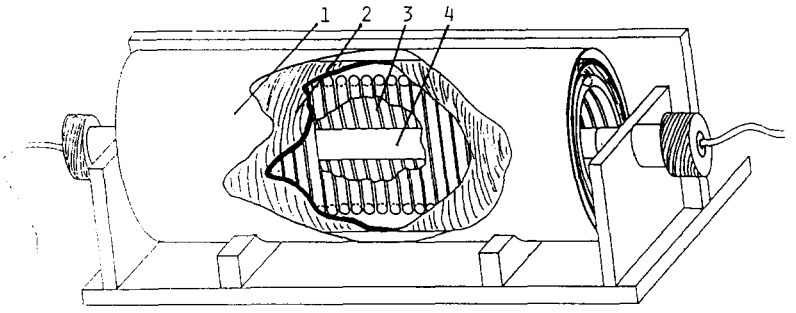

Fig. 2. Construction of the apparatus for high-energy flashed light. Shown is the apparatus with a quartz spiral tube fitted over the flash tube. 1 = outer stainless steel tube, 2 = inner Plexiglas tube, 3 = quartz spiral tube, 4 = ultraviolet tube.

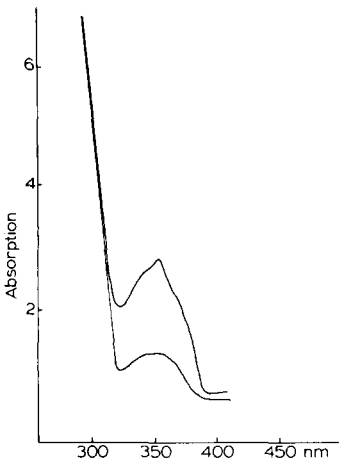

Fig. 3. Long-wave ultraviolet spectra of the diaziridine derivative of cyclosporin A before and after photolysis by ultraviolet light flash. Cyclosporin-diaziridine was dissolved in ethanol. Ultraviolet spectra were recorded before and after a single high-energy ultraviolet light flash.

described has the advantage that aliquots of cell suspensions can be activated after varied periods of preincubation with photoaffinity labels. The time dependence of permeation and distribution of any photoaffinity label can thus be studied. For example, the behavior of [^{3}H]cyclosporin-diaziridine is shown in Fig. 4. The radioactivity of the 85 kDa protein increases during a preincubation of 1–30 min. In other words high energy flash photoaffinity labeling is suitable for the demonstration of dynamic processes which is not possible with continuous ultraviolet light irradiation. Occasionally certain compounds can be activated to covalently binding derivatives (e.g. epoxides) without any previous chemical modification. In our labora-

TABLE I

INFLUENCE OF ULTRAVIOLET LIGHT FLASH ON TRANSPORT FUNCTION AND CELL VIABILITY OF ISOLATED HEPATOCYTES

The uptake of cholate in isolated hepatocytes ($2 \cdot 10^6$ cells/ml Tyrode buffer pH 7.4, 37°C) was measured by addition of 1 μM [^{14}C]cholate plus 5 μM cholate to 1 ml of cell suspension. At varying times, 100 μl were withdrawn and centrifuged through a layer of silicon oil. The radioactivity associated with the cell pellet was measured. The initial uptake rate V_i of cholate (pmol/mg per min) was calculated before and after treatment of cells by a single ultraviolet light (UV) flash. Cell viability was determined by exclusion of Trypan blue (50 μl cell suspension + 450 μl Tyrode buffer plus 50 μl 2% Trypan blue) 1 min after the addition of Trypan blue the percentage of cells excluding the dye was determined in a Bürker-Türk chamber. Results are presented as means ± S.D. ($n = 4$).

	Cholate uptake (pmol/mg per min)	% Trypan blue exclusion
Before UV flash	160 ± 34	77.8 ± 18
After UV flash	152 ± 69	63.8 ± 15

TABLE II

PHOTOAFFINITY LABELING OF RAT HEPATOCYTES WITH VARIOUS RADIOACTIVE LABELS DERIVED FROM BILE ACIDS OR FROM CYCLOSPORIN A

Photoaffinity label	Molecular mass of labeled membrane proteins (kDa)	
	strong labeling	weak labeling
Azido[^{14}C]benzamido-taurocholate	50–54	37
[^{3}H]cyclosporin diaziridine	50–54	85, 34

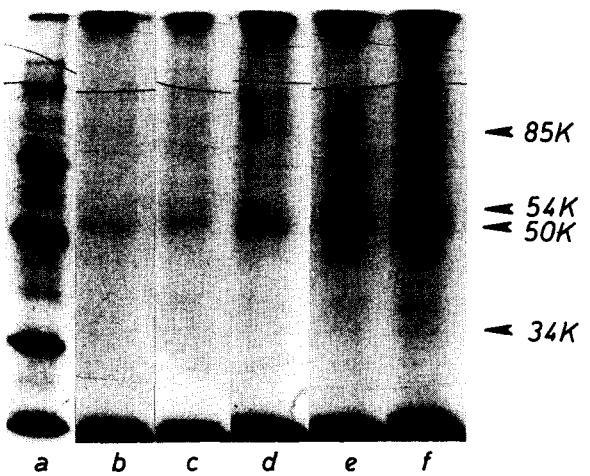

Fig. 4. Time dependence of photoaffinity labeling of isolated hepatocytes by [^{3}H]cyclosporin-diaziridine. Isolated hepatocytes ($2 \cdot 10^6$/3 ml Tyrode buffer (pH 7.4)) were preincubated 1 (b), 5 (c), 10 (d), 15 (e), 30 (f) min in the dark with 5.6 μM of [^{3}H]cyclosporin-diaziridine before treatment with a single ultraviolet light flash. The labeled proteins of isolated hepatocytes were separated by SDS gel electrophoresis. The labeled proteins were visualized by fluorography of SDS slab gels. (a) Standard proteins.

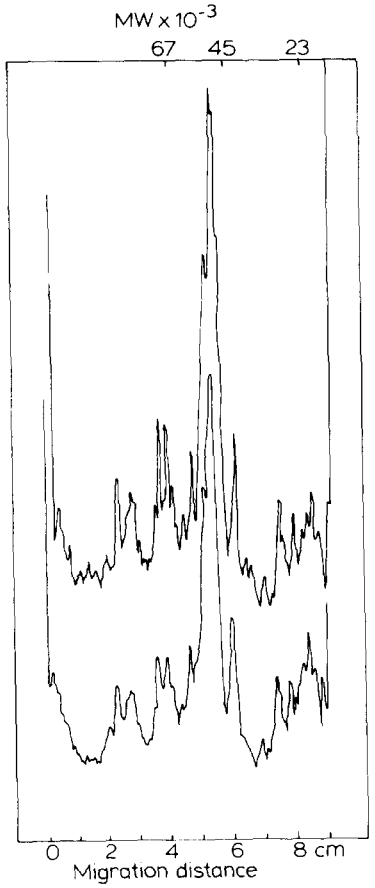

Fig. 5. Protein pattern of isolated plasma membranes before and after a single ultraviolet light flash. Isolated rat liver plasma membranes (0.3 mg/ml phosphate-buffered saline (pH 7.4)) were treated with a single ultraviolet light flash at room temperature. Plasma membrane proteins were separated by SDS gel electrophoresis. The protein pattern of ultraviolet light-treated and untreated membranes was scanned at 570 nm. Upper curve: control plasma membranes; lower curve: ultraviolet light-treated plasma membranes.

tory, isolated hepatocytes were directly photoaffinity labeled with [^{3}H]aflatoxin (unpublished).

Discussion

Several types of mercury and xenon lamps were used for photoaffinity labeling of biological material [1]. The application of flash lamps has not been common in biological experiments [2]. Commercial instruments may be often too expensive for occasional use. The equipment described here is practicable in many photoaffinity labeling experiments on isolated membranes as well as on whole cells. Apart from the experiments described here with rat hepatocytes, we have also labeled erythrocytes, lymphocytes and brush-border membranes with cyclosporin A diaziridine, by application of a single flash.

In our experience marked changes in the protein pattern of membranes (loss of proteins or formation of high molecular weight aggregates) was not observed after treatment with single flashes (Fig. 5) (for review see Ruoho et al. [1]). We feel that protective procedures such as prevention of photooxidation by bubbling argon, prevention of amino acid destruction by strong filters, or prevention of denaturation of protein by cooling are not needed. Covalent coupling of activated reactants will probably occur earlier than breakdown of biological structures. In all cases in which functions of cells or cell organelles must be tested after photoaffinity labeling the experimental conditions must be modified so that functional damage remains tolerable. The use of several radical scavengers for cell protection during irradiation were not successful in our experiments. Thus far, adequately efficient filters are not available to protect certain sensitive structures, however quartz cuvettes can be filled with absorbing solutions and adapted so that only the actual photolyzing wavelength can pass.

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