

# Spontaneous Release of Malondialdehyde from Ultraviolet Light Exposed Liposomal Membranes

Sanjiv Agarwal, Anusua Ghosh, and S. N. Chatterjee

Biophysics Division, Saha Institute of Nuclear Physics, 37 Belgachia Road, Calcutta-700037, India

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UV absorption spectroscopy revealed the presence of MDA with characteristic maximum at about 266 nm in the dialysate of the UV irradiated liposomal suspension. The amount of MDA released increased linearly with increasing UV dose. The fluorescence method for the assay of MDA was found nearly 1500-fold more sensitive than the TBA colour reaction method.

## Introduction

Earlier studies reported by us [1–4] and also by others [5–7] revealed that the ultraviolet light (UV light) exposure produces lipid peroxidation and malondialdehyde (MDA) in the liposomal membrane. In these studies MDA was detected from the characteristic colour (535 nm) that resulted after reaction of the whole membrane suspension with thiobarbituric acid (TBA) at 90 °C and estimated after addition of chloroform and extraction of the colour in the aqueous phase. MDA prepared by acid hydrolysis of tetraethoxypropane has been found biologically active and bind with DNA leading to mutagenesis and carcinogenesis [8–10]. In a natural system the MDA produced in the membrane can bind with DNA provided it is released in the aqueous medium. To our knowledge very little evidence is available in the literature to establish that the MDA produced in the membrane can be released in the medium without heating or any other enzymatic reaction. This study reports in this context that MDA produced in the liposomal membrane by exposure to UV light is spontaneously released and dialysable.

## Material and Methods

Egg lecithin or phosphatidylcholine (obtained from V. P. Chest Institute, Delhi) containing, phosphatidylcholine (67%), phosphatidylethanolamine (20%), lysophosphatidylethanolamine (7%), lysophosphatidylcholine (5%) and sphingomyelin (traces), was used for the preparation of liposomes in

50 mM tris-maleate buffer, pH 5.8 by the method described previously [1–4].

Suitable aliquots of liposomal suspension were taken in plastic petridish (diameter = 35 mm) to a depth of about 1 mm and then exposed to UV light (254 nm) in presence of atmospheric oxygen under shaking. UV radiation dose was measured by ferri-oxalate actinometric procedure [11].

Usually 1 ml of irradiated or unirradiated liposomal preparation was dialysed overnight (unless otherwise mentioned) against 10 ml of 50 mM tris-maleate buffer, pH 5.8. 2 ml of the dialysate was taken and diluted to 4 ml with 50 mM tris-maleate buffer, pH 5.8, 1 ml of 1:1 (v/v) mixture of thiobarbituric acid (TBA) and glacial acetic acid was added and the tubes were placed in boiling water bath for 60 min. Tubes were cooled down to room temperature and fluorescent MDA-TBA adducts were extracted with 5 ml butanol in the butanol layer [12]. Fluorescence intensities were measured on Shimadzu Recording Spectrofluorophotometer, Model RF-540. The actual amount of MDA was determined from a calibration curve plotted with the help of known concentration of standard MDA prepared by the hydrolysis of 1,1,3,3-tetraethoxypropane [13]. MDA in the dialysate was also measured by absorption spectroscopy of MDA-TBA adduct, following in general the method of Placer *et al.* [14] as described earlier [2, 4].

## Results

The dialysate obtained after overnight dialysis of irradiated and also unirradiated liposomal suspensions were subjected to UV absorption spectroscopy, the dialysate of the unirradiated sample being used as reference. Fig. 1 shows the absorption spectrum

Reprint requests to S. N. Chatterjee.

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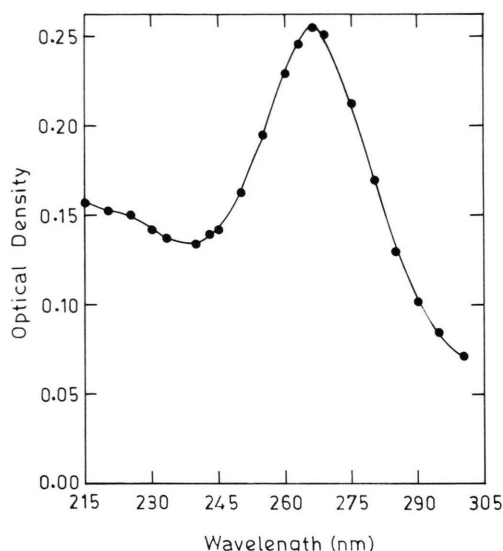


Fig. 1. UV exposed liposomes were dialysed overnight. The figure shows the absorption spectrum of the untreated dialysate. UV dose,  $8.11 \times 10^4$  ergs/mm<sup>2</sup>.

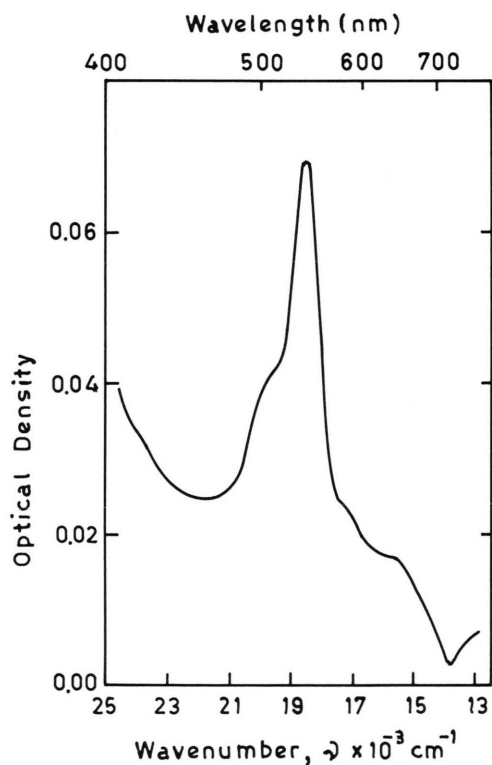


Fig. 2. UV exposed liposomes were dialysed overnight and the dialysate subjected to TBA reaction. The figure shows the absorption spectrum of MDA-TBA adduct in the dialysate. UV dose,  $8.11 \times 10^4$  ergs/mm<sup>2</sup>.

with an absorption maximum at about 266 nm, which is characteristic of MDA [13]. No significant amount of conjugated dienes or trienes was detected. Also colour reaction with iodide was insignificant indicating absence of hydroperoxides in the dialysate. When the dialysate was subjected to TBA reaction, UV absorption spectroscopy revealed a maximum at about 535 nm (Fig. 2). Also the fluorescence spectroscopy of the dialysate after TBA reaction revealed the presence of an emission maximum at about 550 nm corresponding to an excitation maximum at 540 nm (Fig. 3). Spectroscopic evidences thus confirmed the formation of MDA-TBA adduct in the TBA reacted dialysate [12, 13, 15]. A considerable amount of MDA produced in the liposomal membrane by UV exposure was released in the aqueous medium and could be dialysed. The amount of MDA available in the dialysate increased with increasing time of dialysis and attained a plateau from 5 h onward (Fig. 4). Also the MDA released in the aque-

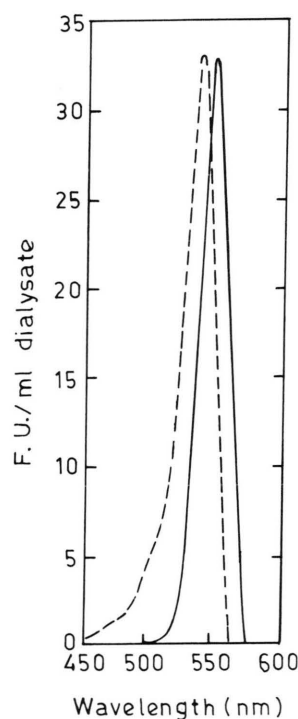


Fig. 3. UV exposed liposomes were dialysed overnight and the dialysate subjected to TBA reaction. The figure shows the fluorescence excitation and emission spectra of the MDA-TBA adduct formed in the dialysate. UV dose,  $8.11 \times 10^4$  ergs/mm<sup>2</sup>.

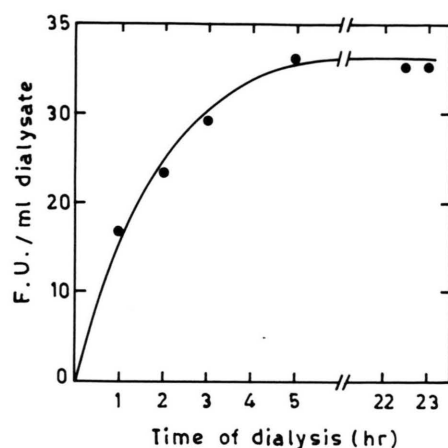


Fig. 4. Release of increasing amounts of MDA in the dialysate with increasing period of dialysis of the liposomal preparation exposed to UV dose of  $8.11 \times 10^4$  ergs/mm<sup>2</sup>. The dialysate was subjected to TBA reaction and the fluorescence emission of MDA-TBA adduct was measured at 550 nm, the excitation wavelength being 540 nm.

ous medium and available in the dialysate increased linearly with increasing UV dose. MDA was estimated by both fluorescence and absorbance spectroscopic methods and both the methods were in reasonable quantitative agreement with each other, as shown in Fig. 5. However, when the respective

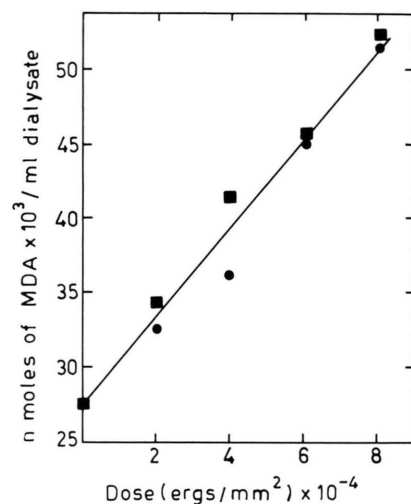


Fig. 5. The increasing amount of MDA available in the dialysate (overnight) with increasing dose of UV. MDA was estimated either by colorimetry at 535 nm (■—■) or by fluorescence analysis at 550 nm (●—●).

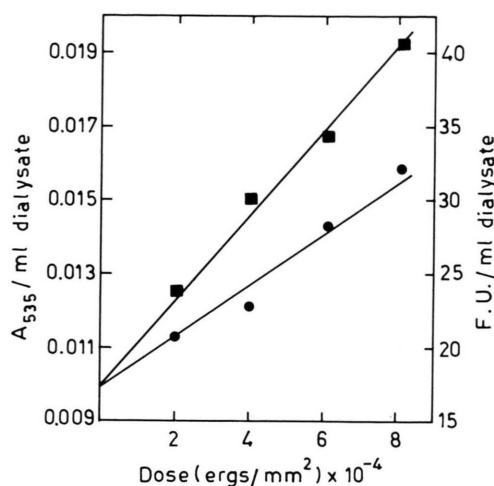


Fig. 6. The data of Fig. 5 are plotted with the ordinate scale either in terms of fluorescence units [F.U.]/ml dialysate (●—●) or in terms of  $A_{535}$ /ml dialysate (■—■) against the UV dose.

data were plotted with the absorbance units or fluorescence units as ordinate against the UV dose, two different straight lines having different slopes were obtained indicating that the two methods exhibited different order of sensitivity (Fig. 6). The slopes  $S_1$  ( $\Delta A_{535}/\Delta \text{dose}$ ) and  $S_2$  ( $\Delta \text{F.U.}/\Delta \text{dose}$ ) were estimated as 0.00115 and 1.6875, respectively. The value of  $S_2/S_1$  was 1467.4 indicating that the fluorescence method was nearly 1500 times more sensitive than the absorbance spectroscopic method in detecting the MDA.

## Discussion

Radiations and chemicals have been shown to induce lipid peroxidation in liposomes and biological membranes [1–7]. The permeability of the membrane has also been shown to increase in parallel with the lipid peroxidation indicating that the interior of the membrane undergoes alterations [1, 2, 4]. MDA is usually considered to be an important end-product of lipid peroxidation [9, 15]. It has been claimed that MDA is mutagenic and carcinogenic [8–10]. The different stages in the production of MDA by free radical reactions leading to alterations and/or rearrangement of chemical bonds in the polyunsaturated fatty acid chains have been postulated by different workers [9, 15]. Although the scheme of

free radical reaction leading to lipid peroxidation indicates that MDA once formed is detached from the main fatty acid chain, not much experimental evidence is available as to whether the MDA will be spontaneously available outside the membrane in the intracellular or extracellular fluid. Pryor *et al.* [15] have postulated that MDA will be detached from the fatty acid chain by thermolytic or enzymatic process. If MDA can be released without the thermal cleavage of the endoperoxide or without any enzymatic mechanism, the process acquires greater significance or importance. It could then be more frequently and universally available for interaction with DNA leading to mutagenesis or carcinogenesis. Nakazawa *et al.* [16] produced evidence of the release of MDA in the surrounding medium by taking recourse to ultracentrifugation at  $105\,000\times g$  for 1 h of the  $\gamma$ -irradiated liposomal suspension. Since liposomal material often exhibits considerable heterogeneity in size and a small density difference from the aqueous medium, all the liposomal particles might not be pelleted by such centrifugal procedure. Accordingly the TBA reactive substance in the supernatant may not reveal the presence of free MDA only. In the present set of experiments these possibilities were eliminated.

There is practically no chance of the dialysate being contaminated with any of the liposomes. Also the untreated dialysate revealed the characteristic absorption maximum (266 nm) of MDA. The present set of experiments thus establish while confirming at the same time the observations of Nakazawa *et al.* [16] that MDA is released in the surrounding aqueous medium. It is further interesting to note that the dialysis experiment also reveals a linear dose response relationship as was demonstrated earlier with the whole liposomal suspension [1–4]. It is also satisfying to note that the MDA available in the dialysate exhibited the absorption and fluorescence excitation-emission spectra [12] characteristic of the MDA-TBA adduct.

In the present set of experiments, the presence of MDA in the dialysate was confirmed by the UV absorption spectroscopy. It was quantitatively estimated by two methods in parallel, the fluoremetric and the absorption spectroscopic methods. Both the methods agreed with each other in so far as quantitative assay of MDA was concerned. However, the fluoremetric assay was found at least 1500 times more sensitive than the absorption spectroscopic method.

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