Biochimica et Biophysica Acta 905 (1987) 227–230
Elsevier

BBA 70536

BBA Report

An evaluation of the contribution of membrane lipids to protection against ultraviolet radiation

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(Received 10 July 1987)

Key words: Ultraviolet radiation protection; Membrane lipid;(*E. coli*); (Vesicular stomatitis virus)

Using dioleoylphosphatidylcholine liposomes incorporating various fatty acids and neutral lipids, we have examined the ability of such lipids to provide protection of *Escherichia coli* and vesicular stomatitis virus (VSV) against the lethal effect of ultraviolet (254 nm) radiation. While the presence of varying amounts of saturated (palmitic) or polyunsaturated (arachidonic) fatty acids or the lipid antioxidant, α-tocopherol, had little effect on killing by ultraviolet radiation, considerable radioprotection was observed with β-carotene, retinal and vitamin K-1 at final concentrations of 1 mg/ml. In another approach, vesicular stomatitis virus grown under conditions in which its envelope fatty acid composition was substantially modified, showed little change in its sensitivity to inactivation by ultraviolet radiation. The results provide strong evidence for a radioprotective role of certain, relatively rare natural lipid components with conjugated polyene systems, but not of the more ubiquitous and abundant membrane fatty acids.

Membrane lipids of viruses, prokaryotes and eukaryotes have been considered as both targets of radiation-induced damage as well as potential radioprotective constituents. The contribution of membrane lipid constituent fatty acids to radioprotection remains incompletely clarified. Studies on ionizing radiation with an *Escherichia coli* fatty acid auxotroph suggested a correlation between fatty acid composition and radiation sensitivity [1,2] while data from fatty acid supplemented mycoplasma [3] or mammalian cells [4,5] argued against a role, at least for polyunsaturated fatty acids, in influencing susceptibility to radiation-killing. Little evidence is so far available regarding the possible role of membrane lipids to cellular protection against ultraviolet (UV) radiation.

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Due to evidence implicating an etiological role for solar ultraviolet radiation in human skin aging and cancer, much interest has focussed on the identification and development of compounds which inhibit either direct or indirect mechanisms of ultraviolet-induced cell damage. Potentially harmful reactive oxygen species such as peroxides, hydroxyl radicals, singlet oxygen and superoxide anions are formed upon radiative exposure, including that from ultraviolet, X and gamma sources [6]. Resultant reactions have been shown to kill cells [7], cleave DNA [8] and peroxidize lipids [9]. Among the cell's normal defense mechanisms against reactive oxygen species are considered to be enzymes such as catalase and superoxide dismutase and a variety of biological antioxidants. Among the latter are vitamins A (and related carotenoids) and E. Carotenoids are able to quench singlet oxygen [10] and oxidative free radicals [11,12] which have been implicated in cell destruction [7] and tumor promotion [13–16].

Carotenoids have been shown in bacterial systems to be protective against photo-oxidative damage [17,18] and in certain kinds of skin photosensitivity in animals [19,20] and humans [21]. Vitamin E (α-tocopherol) has been shown to be an effective peroxyl radical scavenger which provides significant protection against lipid peroxidation [23,24]. In spite of the above, however, there is an absence of definitive information on the effectiveness of potential lipid radioprotectants on reducing the cell-killing activity or ultraviolet light. We describe here experiments based on the use of phosphatidylcholine vesicles incorporating various lipids in a strategy designed to provide quantitative evaluation of radioprotective activities.

Our first studies were performed using *Escherichia coli* K-12 as radiosensitive target. Suspensions of freshly grown *E. coli*, harvested by centrifugation, washed and resuspended in distilled water were irradiated at room temperature in the absence and presence of dioleoylphosphatidylcholine (DOPC) dispersions containing various lipids suspected of having radioprotective ability. The studies described here involved the use of small unilamellar liposomes prepared by sonication of dried lipid in water [25]. The method of preparation does not seem to be of crucial importance since the use of multilamellar liposomes [26] yielded results very similar to those described here. DOPC itself, at concentrations ranging from 1 to 20 mg/ml showed no significant radioprotection of *E. coli* ($D_{37} = 67$ J/m^2). In contrast, the incorporation into DOPC of either β-carotene ($D_{37} = 120$ J/m^2), vitamin K-1 ($D_{37} = 330$ J/m^2) or retinal ($D_{37} = 170$ J/m^2) at a final concentration of 1 mg/ml provided considerable protection of *E. coli* against ultraviolet-killing (Fig. 1). Surprisingly, α-tocopherol, either in the free form or as the acetate had no protective effect on bacterial survival. Thus, it would seem that the well-documented peroxyl-trapping activities of α-tocopherol [23,24], are not of major importance in protecting cells against the lethal effects of ultraviolet radiation.

Further studies were conducted with the lipid-enveloped, RNA virus, vesicular stomatitis virus (VSV) as target for ultraviolet-inactivation. Vesicular stomatitis virus consists solely of a ribonucleoprotein core (single stranded RNA genome associated with three polypeptides) surrounded by a bilamellar lipid envelope (containing one internally situated peripheral membrane polypeptide and one integral glycoprotein). Compared to membranes of higher organisms, the vesicular stomatitis virus envelope is remarkably simple and homogeneous in structure. Moreover, there are no repair functions encoded by the vesicular stomatitis virus; as a consequence damage to the RNA genome will not be obscured by post-irradiation repair process, and may therefore be reliably scored by simply assay of virus infectivity. Vesicular stomatitis virus was irradiated in the presence of vesicles of DOPC (10 mg/ml), some preparations of which contained the following supplements (final concentration of 1 mg/ml): palmitic acid (C16 : 0), arachidonic acid (C20 : 4) or β-carotene. As shown in Fig. 2, vesicular stomatitis virus irradiated in the presence of DOPC alone was inactivated at a D_{37} of 12 J/m^2. The inclusion of 10% w/w arachidonic acid in the DOPC vesicles had an insignificant effect on the radiosensitivity of vesicular stomatitis virus ($D_{37} = 12$ J/m^2), whereas the presence of 10% w/w palmitic acid resulted in a slight increased radiosensitivity ($D_{37} = 10$ J/m^2). DOPC vesicles containing 10% w/w

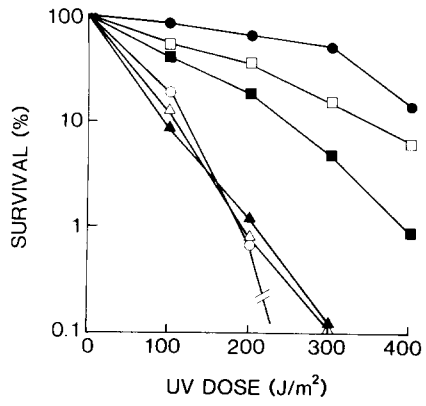

Fig. 1. Survival of *E. coli* irradiated in the presence of DOPC vesicles containing potential lipid radioprotectants. Freshly harvested and water-washed cells of *E. coli* (10^7 colony forming units) were irradiated at room temperature with ultraviolet (UV) light (254 nm) in wells of a 24-well plate (Nunc plastics) containing DOPC vesicles (5 mg/ml). DOPC vesicles were either unsupplemented (○) or supplemented with 20% w/w vitamin K-1 (●), retinal (□), β-carotene (■), α-tocopherol (△) or α-tocopherol acetate (▲). At intervals of 20 s, aliquots of 10 μl were removed for quantitation of colony forming units by plating in nutrient agar (Difco).

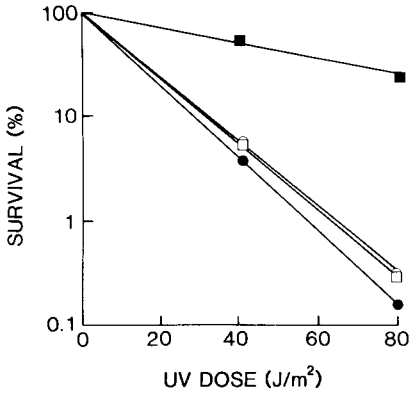

Fig. 2. Effect of saturated and unsaturated fatty acid on ultraviolet (UV) radiation sensitivity of vesicular stomatitis virus. Freshly pelleted and water-washed vesicular stomatitis virus (10^8 plaque forming units) was irradiated as above in the presence of DOPC vesicles (10 mg/ml). DOPC vesicles were either unsupplemented (○) or supplemented with 10% w/w C16:0 (●), C20:4 (□) or β-carotene (■). At intervals of 10 s, aliquots of 10 μl were removed for quantitation of plaque forming units on mouse L cells.

β-carotene, on the other hand, provided a marked degree of radioprotection ($D_{37} = 49$ J/m^2). The results clearly show that the naturally occurring unsaturated fatty acids (e.g. C20:4), at concentrations in which they are commonly found in biological membranes, have little effect on moderating the radiation sensitivity of the enclosed cell or genome. In contrast, the presence in cell membranes of lipid constituents such as β-carotene may have a profound effect on radiosensitivity. Within the context of the latter finding, it is worth noting that diet-supplemented β-carotene has been reported to deposit in the epidermis of mice and contribute to reduced tumor incidence and the quenching of reactive oxygen species [27,28].

It has been shown previously that culture of mouse fibroblast LM cells in media supplemented with fatty acids results in the modification of cell membrane fatty acid composition [29,30]. It has also been shown that manipulation of the lipid composition of cultured cells is reflected in the lipid envelopes of virions which bud through cellular membranes [31,32]. We used a similar approach to produce vesicular stomatitis virus with varied fatty acid compositions. Vesicular stomatitis virus was grown in mouse fibroblast LM-K cells [33] which had been supplemented with either palmitic acid (C16:0) or arachidonic acid (C20:4) complexed to delipidated bovine serum albumin. As shown in Table I, the fatty acid composition of vesicular stomatitis virus produced from supplemented and non-supplemented LM-K cells varied considerably, as reflected by a range from 0.83 to 1.70 in the average number of double bonds/mol of fatty acid. Vesicular stomatitis virus, in which the lipid envelope was enriched in unsaturated fatty acids (grown in C20:4-supplemented LM-K cells) shows an ultraviolet-inactivation curve which was indistinguishable from that of vesicular stomatitis virus grown under non-supplemented

TABLE I

ULTRAVIOLET-LIGHT SENSITIVITY OF VESICULAR STOMATITIS VIRUS WITH MODIFIED ENVELOPE LIPID COMPOSITION

Cultures of pregrown, confluent LM-K cells were incubated for 24-h in minimal essential medium containing bovine serum albumin, bovine serum albumin complexed with C16:0, or bovine serum albumin complexed with C20:4. Bovine serum albumin was present at 0.42 mg/ml; concentrations of C16:0 or C20:4 fatty acids were 3.7 μg/ml. After the 24-h incubation period, cells were inoculated with vesicular stomatitis virus at a multiplicity of 10 plaque forming units/cell and vesicular stomatitis virus was harvested by centrifugation (60 min, 100000 × g) of the supernatant medium. Virion lipids were extracted with chloroform/methanol (1:1, v/v) and lipid extracts treated with 5% methanolic HCl (1 h at 70°C) to liberate fatty acid methyl esters which were subsequently identified and quantitated by integrative gas chromatography. Pelleted virions were washed and resuspended in distilled water at a concentration of 10^8 plaque forming units/ml and irradiated as described in the legend to Fig. 2.

Fatty acid	mol% fatty acid in vesicular stomatitis virus		
	unsupplemented	C16:0 supplemented	C20:4 supplemented
C-14:0	4.48	4.60	3.08
C-16:0	19.80	30.30	18.02
C-16:1	6.08	5.69	1.47
C-18:0	20.39	20.11	28.32
C-18:1	10.06	7.85	3.12
C-18:2	35.48	28.02	9.06
C-20:4	2.76	2.33	25.10
C-22:4	0.95	1.10	11.83
Double bonds/mol fatty acid	1.02	0.83	1.70
D_{37} (J/m^2)	12	11	12

conditions ($D_{37} = 12$ J/m^2). By comparison, vesicular stomatitis virus which was enriched in saturated fatty acids (grown in C16 : 0-supplemented cells) had a somewhat increased sensitivity to ultraviolet light ($D_{37} = 11$ J/m^2). It is apparent from this study that naturally occurring fatty acids, in concentrations at which they may be normaly present in the vesicular stomatitis virus lipid envelope, are not likely to play a significant role in limiting ultraviolet damage to the RNA genome.

Taken together, the results of the above studies strongly argue that biological killing by ultraviolet radiation may be significantly moderated by the presence of relatively rare membrane lipid components, such as β-carotene, retinal or vitamin K, while the more ubiquitous and abundant membrane fatty acids have a negligible contribution. It is also clear that compounds (e.g. α-tocopherol) which are effective inhibitors of lipid peroxidation [23,24] do not necessarily provide protection against oxidative cell-killing processes generated during ultraviolet irradiation.

The support of the Medical Research Council of Canada is gratefully acknowledged. R.A. is a Scholar of the Alberta Heritage Foundation for Medical Research.

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