Correlation between Microwave-Induced Lipid Peroxidation and Liposome Leakage

Melánia Babincová
Department of Biophysics, Comenius University, MFF UK, Mlynská dolina, 842 15 Bratislava, Slovak Republic

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A direct correlation has been found for the microwave-induced phosphatidylcholine peroxidation and 6-carboxy-fluorescein leakage from liposomes.

Introduction

Recently it has been suggested that biological membranes and more specifically phospholipids in natural membranes may represent the major sites of interaction with microwave radiation [1–3]. The nature of the physical or chemical interactions responsible for a non-thermal influence of microwave radiation on biological membranes is not yet known. Ionizing radiation, *e.g.* γ-rays, X-rays, α-particles and non-ionizing radiation, *e.g.* ultraviolet light and ultrasonic irradiation were shown to initiate lipid peroxidation in liposomal membrane [4].

In this work we investigate the correlation of microwave-induced lipid peroxidation and leakage of dye 6-carboxy-fluorescein from liposomes.

Materials and Methods

Lipid-egg phosphatidylcholine (Sigma, U.S.A.) was dissolved in organic solvents (a mixture of chloroform and ethanol 2 : 1, v/v). The lipid solution was evaporated at vacuum in a rotary evaporator. After evaporation of the solvent, Tris buffer (pH = 7.4) containing 0.1 M 6-carboxy-fluorescein was added into the glass vessel with lipid film, and solution was shaked up mechanically. This suspension was then sonicated with Brown Labsonic 2000 sonicator at 80 W for 15 min under nitrogen atmosphere in an ice-bath, to obtain small unilamellar liposomes. Liposomes were separated from free 6-carboxy-fluorescein and resuspended in Tris buffer to obtain the final lipid concentration of 3.1 mg/ml.

Reprint requests to M. Babincová.

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Microwave radiation was generated by a continuous wave klystron (20 SR 51, Tesla, Czech Republic) operating at 2.45 GHz. The klystron was coupled to waveguide which was terminated with a horn antenna with an aperture with the dimensions of 157 mm × 135 mm in the E and H planes, respectively. All irradiations were performed at a distance of 30 cm. The sample container was made from teflon and was placed inside a polymethylcrylate cell through which water was circulated from a thermostated bath placed outside the radiation field. Stirring of the sample itself was achieved by a slow flow of nitrogen bubbles *via* a small plastic tube protruding through the lid of the sample container. The temperature was monitored continuously by a non-absorbing Vitek thermistor probe into the sample volume. Irradiation was performed at 20 °C, temperature fluctuated less then 2 °C. Absorbed power of the microwave radiation was determined as follows: The sample was exposed to radiation for about 5–15 min without sample thermostating and the temperature of the sample was recorded as function of the time of exposure. Specific absorbed power (SAP) was calculated using expression $SAP = C_p \Delta T/\Delta t$, where C_p is the heat capacity and $\Delta T/\Delta t$ is initial slope of the temperature *versus* time dependence. The specific absorbed power in our experiment was SAP = 490 ± 20 mW/g (we have used C_p = 4200 J/kg).

Absorption spectra of conjugated dienes were recorded in the wavelength range 215–320 nm by a SPECORD M-20 (Zeiss, Jena) UV-VIS spectrophotometer. The increase of the absorption at 233 nm was taken as an indication of the appearance of conjugated dienes and the oxidation index was evaluated from the ratio of the absorbances (A_{233}/A_{215}) [5, 6].

The liposome leakage was studied by determination of the fluorescence increase due to the release and subsequent dilution of the liposome-entrapped, self-quenching probe 6-carboxy-fluorescein (Eastman Kodak Company, Rochester). Fluorescence intensity I was measured at 520 nm (excitation at 490 nm) with Perkin-Elmer MPF-3 L fluorimeter. Percentage increase of dye leakage was determined as $[(I-I_{min})/(I_{max}-I_{min})] \times 100$ (%), where I_{min} is minimum fluorescence intensity given by a nonexposed sample and I_{max} is maximum fluorescence intensity given by a sample after total desintegration of liposome structure by Triton X-100.

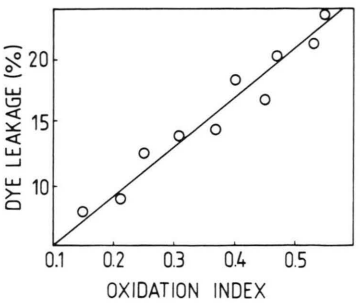

Fig. 1. Dependence of leakage of the dye 6-carboxy-fluorescein from liposomes as a function of the oxidation index A_{233}/A_{215}.

Results and Discussion

Previously, the non-thermal influence of microwave radiation on membrane permeability has been studied [1, 2]. As a mechanism for the observed enhanced permeability it has been proposed that local dissipation of microwave energy leading to a membrane disruption due to the formation of transient pores. Another interpretation is based on the fact that introduction of microwaves may excite a gauche-*trans* isomerization of acyl chains and may, through the excitation of water, which is a strong microwave absorber, facilitate the hydration of polar head groups, leading to physical changes associated with increased permeability [1]. Due to the fact that peroxidation products increase the degree of disorder in lipid bilayer and affect permeability [7] and fluidity [8] of the liposomes, lipid peroxidation may be the mechanism of non-thermal influence of microwave radiation on lipid membranes. As a result of peroxidation non-lamellar phases occur in lipid bilayers [9, 10], which may lead to membrane destabilization and liposome leakage.

To verify this hypothesis we have measured 6-carboxy-fluorescein leakage and corresponding lipid peroxidation of a given liposome sample after different times of exposure to microwave radiation (ranging from 0 to 120 min). We have found an almost linear dependence of the percentage of dye leakage on the oxidation index as shown on Fig. 1.

Our studies confirm the opinion that lipid components in complex biological systems are the target for the microwave radiation and indicate direct a relationship between lipid peroxidation and microwave-induced membrane damaging.

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