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Action of ionizing radiation on lipid-protein interaction[2]

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Laser Raman spectroscopy has been used to investigate the effect of ^{137}Cs γ-rays (100 rad) on the interaction of proteolipid apoprotein in liposomes prepared from dipalmitoyl phosphatidylcholine + linoleic acid. Thermal transitions have been derived from the temperature-dependent CH-stretching features of the lipids. The low temperature transition ($T_c \sim 14^o$) assigned to a proteolipid apoprotein-linoleic acid phase, disappears upon irradiation (2 h after 100 rad). The mid-temperature transition ($T_c \sim 32^o$), assigned to a dipalmitoylphosphatidyl choline-proteolipid apoprotein phase, remains almost unchanged after $\sim$ 2 h of 100 rad ^{137}Cs. We postulate that ionizing radiation preferentially alters the protein-bound linoleic acid-rich phase.

INTRODUCTION

There is evidence that membranes act as a cellular radiation target (1-3). Membrane irradiation yields oxidation fragments of unsaturated lipids (4) which can react with proteins and may change the nature of lipid-protein interaction.

Proteolipids are integral membrane proteins found in myelin (5), mitochondria (6) and sarcoplasmic reticulum (7). We have selected proteolipid apoprotein (PLA) of myelin (5) as a model to investigate the effect of radiation on lipid-protein interactions. Our choice is based on the observation that PLA associates preferentially with phospholipid phases enriched in linoleic acid (8). This may mean that PLA bears sites that preferentially associate with kinks and/or regions of slightly lower hydrophobicity created by cis-double bonds (8). These possibilities are of interest in terms of the peroxidation of linoleate to hydro-

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[2] Abbreviations used: PLA, proteolipid apoprotein; DPL, dipalmitoylphosphatidylcholine; LA, linoleic acid; 13-LA, 13-hydroperoxide of linoleic acid.

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peroxides and make PLA useful for the study of radiation effects on artificial lipid-protein membranes.

METHODS

Synthetic DPL and LA were obtained from Sigma (St. Louis, MO). Lipid-free proteolipid apoprotein (PLA) from brain white matter was prepared as in (9). Multilayered liposomes of DPL + LA (1/0.6 M/M) ± PLA were prepared as follows: Stock solutions of PLA (4 mg/ml) in chloroform-methanol (2/1 V/V) were mixed with DPL ± LA (total lipid/protein, 20/1, w/w). The solvent was removed by N_2 gas, followed by desiccating under vacuum for 2-4 h. The samples were hydrated with ^2H_2O-H_2O (1/1,V/V) and were kept in a water bath at 40±2° for 1 h. Equilibration at temperatures greater than 45° was avoided because these produce a high background in the Raman spectrum probably due to PLA denaturation.

The liposomes, sealed in Kimax capillaries, were irradiated (100 rad, using a with ^{137}Cs irradiator, (Mark II, Sheppard Associates) at a dose rate of 5 rad/min at room temperature. Radiation studies were repeated at least on four different sample preparations.

Raman spectra were recorded using a Spex Ramacomp 4 spectrometer (Spex Industries) modified for use with an optical multichannel analyzer (Princeton Applied Research, NJ). The M6 mirror, (0.85 m) of the second monochromator has been replaced by a 1 m mirror. The movable middle slit has been replaced by a 12.7 x 5.2 mm fixed slit and baffles milled into an aluminum block of the middle slit dimensions. The exit slit has been removed. Both 1200 gr/mm gratings have been replaced by 600 gr/mm gratings. An additional filter has been inserted before the entrance slit to minimize Rayleigh-scattered light.

The detector, a PAR 1205D silicon intensified target tube (SIT) is housed in a PAR 1212 cooled housing. The PAR 1212 unit is rigidly attached to the exit port of the monochromator by an adapter adjusted to focus the Raman-scattered light onto the face of the SIT tube. The tube is precisely oriented in the optical axis. The output of the SIT is detected by a PAR 1205A optical multichannel analyzer once the SIT tube is cooled to ~ -50°, (~ 2h.).

To subtract background signals from sample signals, we match counts at the 0-channel: The detector output with the sample in place is stored in memory A of the 1205A. The sample capillary is then displaced to a position where there is no membrane material in the beam and the Raman spectrum is recorded and stored in memory B of the 1205A analyzer. The difference A-B (projected on a Tektronix display scope) is plotted on an X-Y recorder. The subtraction procedure results in reproducible base lines and Raman features. We collect 1000 scans (32.8 msec each) per control and data point (i.e., ~ 50 sec each). One difference data point takes about 2.5 min. For temperature scans we collect 1000 scans of the CH-stretching region (2800-3000 cm^{-1}) per degree going from -15° to +45° and vice versa. Control and irradiated samples are treated identically and read within 2.5 h of each other.

RESULTS AND DISCUSSION

We have collected the Raman spectra of liposomes composed of DPL + LA ± PLA (control and irradiated) in the CH-stretching region(2800-3000 cm^{-1}) as a function of temperature between -15° and 45°. The liposomes yield three strong bands at 2850, 2880 and 2930±2 cm^{-1} (Fig. 1), previously assigned to symmetric methylene stretching, asymmetric methylene stretching and asymmetric methylene stretching superimposed on symmetric methyl stretching contributions respectively (10). The intensity of the 2880 cm^{-1} feature decreases with a rise in temperature, while that of the 2850 cm^{-1} band remains almost constant.

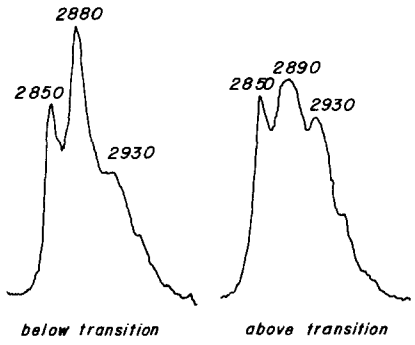

Figure 1. OMA Raman spectra of the 2800-3000 cm^{-1} (C-H stretching) region of liposomes composed of DPL + LA + PLA at various temperatures. Data given for controls and samples irradiated with 100 rad ^{137}Cs γ-rays. (Irradiation at $\sim 21^{o}$).

Thermal transition curves were constructed by plotting $[I_{2880}/I_{2850}]$ vs temperature, where I represents the peak height of respective bands. Fig. 2 demonstrates the thermotropic behavior of DPL + LA $\pm$ PLA $\pm$ irradiation. The curve with open circles shows the phase transition of unirradiated liposomes. The low-temperature transition occurs with onset/completion temperatures at $10^{o}/18^{o}$ (T_c; midpoint $\sim 14^{o}$) and the mid-temperature transition extends from $\sim 27^{o}$ to $\sim 40^{o}$. This transition behavior is equivalent to that presented in a previous publication (8) showing that the low-temperature transition ($T_c \sim 14^{o}$)

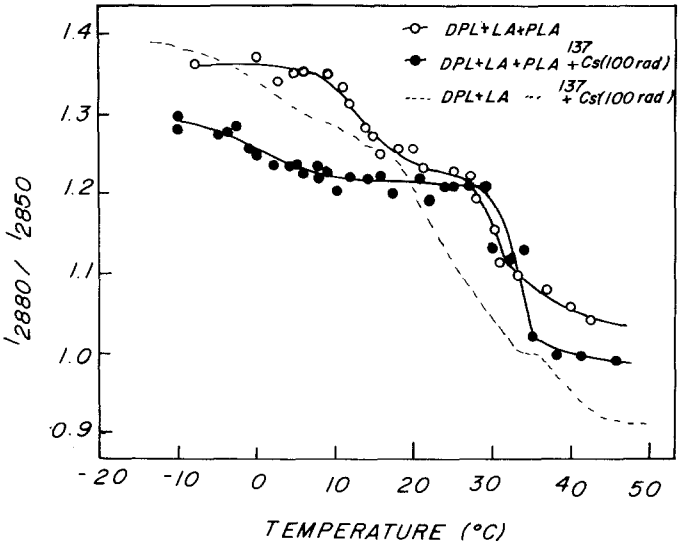

Figure 2. Plots of $[I_{2880}/I_{2850}]$ vs. temperature for liposomes composed DPL + LA + PLA: (○) control ; (●) 2 h after ^{137}Cs (100 rad); (- - -) DPL + LA 2h after ^{137}Cs (100 rad).

represents a phase enriched in PLA-associated LA, whereas the broad mid-tempearture transition represents the DPL-PLA phase.

Raman spectra in the CH-stretching region of irradiated samples were recorded 2 h after exposure to 100 rad ^{137}Cs. The transition curves, given by the solid circles in Fig. 2, show that irradiation abolishes the low-temperature transition (midpoint $\sim 14^{o}$). The mid-temperature transition changes little in terms of the midpoint ($\sim 32^{o}$) but is more cooperative and completes around 35^{o}. These results indicate that the LA-enriched, PLA-containing phase is altered in comparison to the DPL-PLA phase.

The dotted curve in Figure 2 represents the transition behavior of PLA-free DPL liposomes $\pm$ LA 2h after 100 rad of ^{137}Cs γ-rays. The low-temperature transition, which represents the LA phase, widens to $\sim 27^{o}$ with onset/completion temperatures at $\sim -7^{o}/\sim 20^{o}$. The mid-temperature transition ($T_c \sim 26^{o}$ in unirradiated) splits partially, producing a "new" component at $\sim 40^{o}$. This effect is comparable to that obtained by the incorporation of 13-LA (1:0.25 w/w) instead of LA into DPL liposomes. In this case (data not shown) the low-temperature transition (-1 to -4^{o} in DPL+LA) broadens considerably while the mid-temperature transition (onset/completion $+18^{o}/+36^{o}$ in DPL+LA) shifts to $+29^{o}/+40^{o}$. (13-LA does not produce the high-temperature transition observed after irradiation).

We postulate that irradiation generates LA hydroperoxides and that PLA combines with these, resulting in different phase transition characteristics for the system. The experiments with liposomes composed of 13-LA + DPL + PLA liposomes support this view. Infrared spectroscopy (11) has shown that hydroperoxides formed due to the autooxidation of methyl oleate have the trans configuration. As this might also occur in our system, we studied the thermotropic behavior of liposomes composed of linolelaidic acid + DPL + PLA (Fig. 3). The strong, relatively-cooperative low-temperature transition (near 18^{o}) indicates that the interaction of PLA with an acyl chain system containing trans-double bonds differs appreciably from the interaction with a cis-double bond system.

It is possible that PLA itself is damaged by γ-irradiation and that the altered PLA molecule reacts differently with the LA phase. We cannot confirm this at the moment. However, transition curves of liposomes composed of PLA irradiated in

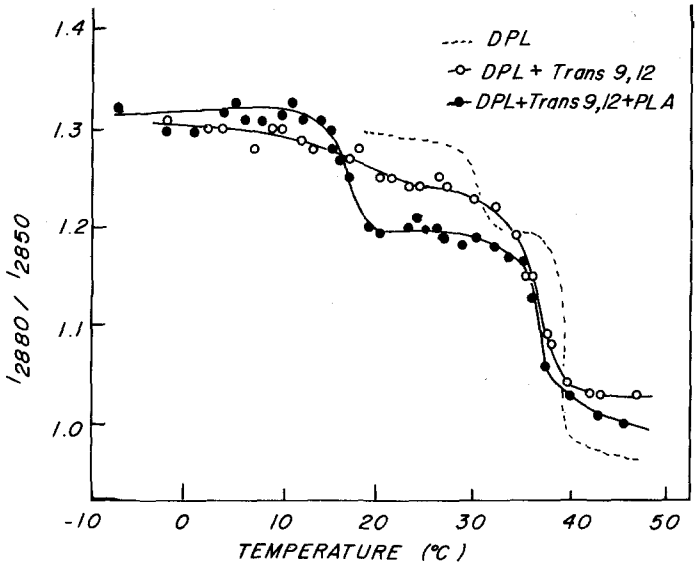

Figure 3. Plots of $[I_{2880}/I_{2850}]$ vs temperature for liposomes composed of DPL ± LA ± PLA. (- - -) DPL; (-o-) DPL/linolelaidic acid (1/0.25; w/w); (●) DPL/linolelaidic acid/PLA (1/0.25/0.06; w/w/w).

chloroform-methanol and unirradiated DPL and LA do not differ significantly from those obtained with unirradiated liposomes (Fig. 2). This information does not exclude the possibility that PLA is more vulnerable to ionizing damage when associated with the lipids and/or that the acyl free radicals produced by irradiation alter the lipid association sites of PLA. We are currently exploring these possibilities.

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REFERENCES

1. Wallach, D.F.H. (1974) in Biomembranes, 5, ed. L. Mauson, Plenum Press, 213-249.
2. Yatvin, M. B., Gipp, J. J. and Dennis, W. H. (1979) J. Radiat. Biol. 25: 539-548.
3. Patrick, J. (1977) in Mammalian Cell Membranes, eds. G. A. Jarneson and D. M. Robinson, Vol. 5, pp. 72-104, Butterworth's, London.
4. Raleigh, J. A., Kremers, W. and Gaboury, B. (1977) Int. J. Radiat. Biol. 31: 203-213.
5. Folch, J. and Lees, M. (1951) J. Biol. Chem. 191: 807-817.
6. Cattel, K. J., Lindop, C. R., Knight, I. G. and Beechey, R. B. (1971) Biochem. J. 125: 169-177.
7. MacLennan, D. H., Yip, C. C. and Iles, G. H. (1977) Cold Spring Harbor Symp. Quant. Biol. 37: 469-477.
8. Verma, S. P., Wallach, D.F.H. and Sakura, J. D. (1980) Biochemistry 19: 574-579.
9. Folch-Pi, J. and Sakura, J. D. (1976) Biochim. Biophys. Acta 427: 410-427.
10. Wallach, D. F. H., Verma, S. P. and Fookson, J. E. (1979) Biochim. Biophys. Acta 559: 153-208.
11. Link, W. E. and Formo, M. D. (1961) in Autoxidation and Antioxidants, Vol. I, Ed. W. O. Lundberg (New York: Interscience) Chapter 10: pp. 397-405.