Ultraviolet A-Induced Peroxidation of Phosphatidylcholine in Unilamellar Liposomes

Azzedine MAZARI, Satoshi IWAMOTO, and Ryo YAMAUCHI

1The United Graduate School of Agricultural Science, Gifu University, Gifu 501-1193, Japan
2Department of Applied Life Science, Faculty of Applied Biological Sciences, Gifu University, Gifu 501-1193, Japan

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Unilamellar liposomes of 1-palmitoyl-2-linoleoyl-3-sn-phosphatidylcholine (PLPC) were exposed to ultraviolet A (UVA) light at 37°C. The UVA-induced PLPC peroxidation was inhibited by free radical scavengers, while singlet oxygen quenchers showed slight inhibition. When pre-existing hydroperoxides were reduced, UVA irradiation could not initiate the lipid peroxidation. The UVA-induced reaction of PLPC hydroperoxides with α-tocopherol produced addition products. The results indicate that UVA irradiation might dissociate hydroperoxides to alkoxyl and peroxyl radicals which stimulate lipid peroxidation.

Key words: ultraviolet A; phosphatidylcholine; lipid peroxidation; liposomes

Ultraviolet A (UVA, 320–400 nm), which is the most abundant UV wavelength of solar radiation reaching the earth, is considered the most important source of oxidative stress in human skin.1 It is capable of penetrating the dermis, producing acute responses such as sunburn and chronic responses such as skin aging, ocular damage, and cancer. Many of the damaging effects by UVA radiation in vivo could be attributed to the peroxidation of membrane lipids.2 UVA-induced lipid peroxidation occurs either through a free radical chain reaction or alternatively through a non-radical pathway by direct reaction with singlet oxygen (\(^1\)O₂). However, the mechanism by which UVA irradiation induces lipid peroxidation is still not clear. We have studied the mechanism of UVA-induced lipid peroxidation by using a unilamellar liposomal system of 1-palmitoyl-2-linoleoyl-3-sn-phosphatidylcholine (PLPC).

PLPC was synthesized as described previously and purified by reversed-phase HPLC.3 PLPC was dispersed in a 10 mM sodium phosphate buffer (pH 7.4) containing 150 mM NaCl, and unilamellar vesicles were made by the extrusion method through a polycarbonate membrane (100 nm in pore size).3 PLPC liposomes (5 mm, 0.50 ml) were put in a Petri dish (2.2 cm in diameter, with a quartz cover) and irradiated at 37°C with an UVA lamp (maximum wavelength at 365 nm, intensity of 22 J/cm² s). PLPC hydroperoxides (PLPCOOH), the primary products of PLPC peroxidation, were quantified by reversed-phase HPLC. To each sample (25 μl) drawn from the liposomes was added methanol (75 μl), and 20 μl of the mixture was injected into a HPLC column. HPLC was conducted in an Inertsil C8-3 column (4.0 x 150 mm, GL Sciences; Tokyo, Japan) with a solvent system of 95% methanol at 0.8 ml/min and detected at 234 nm. The concentration of PLPCOOH was calculated from a standard curve of authentic PLPCOOH prepared from the lipoxygenase-catalyzed peroxidation of PLPC.5 UVA irradiation could accelerate the formation of PLPCOOH in the PLPC liposomal system (Table 1). Some inhibitors were introduced separately to the PLPC liposomes, and their effects on UVA-induced peroxidation were examined. The \(^1\)O₂ quenchers, 1,4-diazabicyclo[2.2.2]octane (DABCO), β-carotene, and sodium azide (NaN₃), slightly inhibited this peroxidation. Exposing liposomes prepared with a 90% deuterium oxide (D₂O) buffer solution to the UVA light revealed no significant increase in PLPCOOH formation. The hydroxyl radical scavenger, thiourea, produced appreciable inhibition of PLPCOOH accumulation, whereas mannitol showed no inhibition. The addition of the metal chelator, diethylenetriaminepentacacetate (DTPA), resulted in no inhibition. On the other hand, the free radical scavengers, butylated hydroxytoluene (BHT) and α-tocopherol, significantly inhibited the PLPC peroxidation.

It has been shown that a free radical-generating system, such as autoxidation, equally yielded two positional isomers of linoleate hydroperoxide (the 9- and 13-isomers), whereas \(^1\)O₂-oxygenation equally yielded the 9-, 10-, 12-, and 13-isomers.6 Thus, the isomeric composition could reflect the mechanism that initiates the peroxidation reactions. The PLPC liposomes were exposed to UVA light for 3 h at 37°C, and the resulting PLPCOOH was isolated by HPLC.7 Mass chromatography of the hydroperoxylinoleate component of PLPCOOH was conducted after reduction, trimethylsilylation, hydrogenation, and trimethylsilylation. Four positional isomers, the 9- (42%), 10- (1%), 12- (2%), and 13-isomers (55%), were present in the resulting trimethylsilyl derivatives of isomeric methyl hydroxyclohexadecanoate, derived from PLPCOOH, by monitoring the fragment ions originating from α-cleavage of the trimethylsilyloxy group (data not

1 To whom correspondence should be addressed. Fax: +81-58-293-2930; E-mail: yamautir@gifu-u.ac.jp

Abbreviations: BHT; butylated hydroxytoluene; DABCO, 1,4-diazabicyclo[2.2.2]octane; DTPA, diethylenetriaminepentacacetate; HPLC, high-performance liquid chromatography; LC-MS, liquid chromatography mass spectrometry; PLPC, 1-palmitoyl-2-linoleoyl-3-sn-phosphatidylcholine; PLPCOOH, 1-palmitoyl-2-linoleoyl-3-sn-phosphatidylcholine hydroperoxides; TPP, triphenylphosphine; UVA, ultraviolet A
However, our result indicates that a minor PLPC in Liposomes (shown). The endogenous photosensitizers are believed to mediate the generation of reactive oxygen species including O\textsubscript{2} when biological samples are exposed to UVA. However, our result indicates that a minor quantity of O\textsubscript{2} have been formed by UVA-irradiation of the liposomes in the absence of a photosensitizer.

Previous studies\cite{10-13} have reported that a trace of pre-existing lipid hydroperoxides in the lipid system might play a major role in UVA-induced PLPC peroxidation. To ascertain the nature of the hydroperoxide-derived free radicals in UVA-induced lipid peroxidation, liposomes (4.0 ml) consisting of PLPCOOH (5 mM) and \(\alpha\)-tocopherol (0.05 mM) were irradiated with UVA light for 15 min at 37°C. A fraction corresponding to the reaction products of PLPCOOH with \(\alpha\)-tocopherol was isolated by preparative HPLC,\cite{7} and analyzed by LC-MS in a Luna C8(2) column (2 x 150 mm) with 10 mM ammonium acetate in methanol at 0.2 ml/min. Compounds were detected at 240 nm. Peaks 1 and 2 were identified to be 1-palmitoyl-2-[9-(8a-dioxy-\(\alpha\)-tocopherone)-12,13-epoxyoctadecenoyl]-3-sn-phosphatidylcholine (9-TOO-epoxyPLPC) and 1-Palmitoyl-2-[9-(8a-dioxy-\(\alpha\)-tocopherone)-10,12-octadecadienoyl]-3-sn-phosphatidylcholine (9-TOO-PLPC).

Table 1. Effect of Inhibitors on the UVA-Induced Peroxidation of PLPC in Liposomes

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (mM)</th>
<th>PLPCOOH formation* ((\mu)M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (no irradiation)</td>
<td></td>
<td>7.7 ± 0.7\textsuperscript{b}</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>44.3 ± 3.7</td>
</tr>
<tr>
<td>DABCO</td>
<td></td>
<td>35.9 ± 14.0</td>
</tr>
<tr>
<td>(\beta)-Carotene</td>
<td>0.005</td>
<td>37.0 ± 7.6</td>
</tr>
<tr>
<td>Na(\textsubscript{2}NO\textsubscript{3}</td>
<td></td>
<td>29.7 ± 8.3</td>
</tr>
<tr>
<td>D2O</td>
<td>90%\textsuperscript{c}</td>
<td>49.3 ± 10.3</td>
</tr>
<tr>
<td>Thiolurea</td>
<td>1</td>
<td>30.4 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>25.0 ± 1.4</td>
</tr>
<tr>
<td>Mannitol</td>
<td>10</td>
<td>43.0 ± 5.4</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>55.0 ± 10.3</td>
</tr>
<tr>
<td>DTPA</td>
<td>1</td>
<td>44.7 ± 1.5</td>
</tr>
<tr>
<td>BHT</td>
<td>0.005</td>
<td>3.0 ± 0.7</td>
</tr>
<tr>
<td>(\alpha)-Tocopherol</td>
<td>0.005</td>
<td>3.3 ± 0.7</td>
</tr>
</tbody>
</table>

* A liposomal suspension of PLPC (5 mM) with or without the indicated inhibitor was exposed to UVA light at 37°C for 3 h.
** Each value is expressed as the mean ± standard deviation of three different experiments.

Liposomes were prepared by using a sodium phosphate buffer solution containing up to 90 vol% of D\(\textsubscript{2}O\) and the concentration of pre-existing PLPCOOH in the present study, the concentration of pre-existing PLPCOOH in PLPC liposomes was determined to be 2.0 ± 0.3 \(\mu\)M. Therefore, the pre-existing hydroperoxides in PLPC liposomes were regulated by treating with triphenylphosphine (TPP) which reduces endogenous hydroperoxides to the corresponding alcohols stoichiometrically.\cite{11} When PLPC liposomes were treated with TPP, this caused the inhibition of UVA-induced lipid peroxidation (Fig. 1). Adding extra PLPCOOH to the TPP-treated liposomes recovered the lipid peroxidation. We deduced that pre-existing lipid hydroperoxides in the liposomal system might play a major role in UVA-induced PLPC peroxidation.

Fig. 1. Effects of TPP and PLPCOOH on the UVA-Induced Peroxidation of PLPC in Liposomes.

Liposomes of PLPC (5 mM) containing TPP and PLPCOOH were irradiated with UVA light for 3 h at 37°C. Each value is expressed as the mean ± standard deviation of three different experiments.

Fig. 2. HPLC Chromatogram of the UVA-Induced Reaction Products of PLPCOOH with \(\alpha\)-Tocopherol and Typical Structures of the 8a-(Lipid-dioxy)-\(\alpha\)-tocopherones, 1-Palmitoyl-2-[9-(8a-dioxy-\(\alpha\)-tocopherone)-12,13-epoxyoctadecenoyl]-3-sn-phosphatidylcholine (9-TOO-epoxyPLPC) and 1-Palmitoyl-2-[9-(8a-dioxy-\(\alpha\)-tocopherone)-10,12-octadecadienoyl]-3-sn-phosphatidylcholine (9-TOO-PLPC).

Liposomes consisting of PLPCOOH (5 mM) and \(\alpha\)-tocopherol (0.05 mM) were irradiated with UVA light for 15 min at 37°C. A fraction corresponding to the reaction products of PLPCOOH with \(\alpha\)-tocopherol was isolated by preparative HPLC,\cite{7} and analyzed by LC-MS in a Luna C8(2) column (2 x 150 mm) with 10 mM ammonium acetate in methanol at 0.2 ml/min. Compounds were detected at 240 nm. Peaks 1 and 2 were identified to be 1-palmitoyl-2-[9-(8a-dioxy-\(\alpha\)-tocopherone)-epoxyoctadecenoyl]-3-sn-phosphatidylcholines and 1-palmitoyl-2-[9-(8a-dioxy-\(\alpha\)-tocopherone)-octadecadienoyl]-3-sn-phosphatidylcholines, respectively.
octadecenoyl]-3-sn-phosphatidylcholines (ESIMS \(m/z\) 1257 ([M + Na]\(^+\)), 807, and 791); and peak 2 was 1-palmitoyl-2-[(8α-dioxy-α-tocopherone)-octadecenoyl]-3-sn-phosphatidylcholines (ESIMS \(m/z\) 1241 ([M + Na]\(^+\)) and 791). These data indicate that the PLPCOOH-derived alkoxy and peroxy radicals were both involved in the UVA-induced decomposition of PLPCOOH.

Since biological membranes always contain a trace amount of pre-formed lipid hydroperoxide (LOOH), UVA is more likely to stimulate peroxidation by LOOH-derived free radicals than by generating \(O_2^+\) or other initiating reactive oxygen species. The UVA-band range can provide sufficient energy to break down LOOH (the photon energy at 365 nm is calculated to be 330 kJ/mol). Photolysis might be initiated by cleavage of the O–O bond in LOOH, which has a weaker bond-dissociation energy of about 180 kJ/mol, to form the alkoxy radical (LO\(^*/\)) (Eq. (1)). On the contrary, the O–H bond in LOOH is relatively more difficult to break, as it requires about 380 kJ/mol of energy (Eq. (2)). The other process is a bimolecular reaction of LOOH which produces both alkoxy and peroxy radicals (Eqs. (2) and (3)).

\[
\begin{align*}
\text{LOOH} & \rightarrow \text{LO}^* + \text{OH}^* \\
\text{LOOH} & \rightarrow \text{LOO}^* + \text{H}^* \\
2\text{LOOH} & \rightarrow \text{LO}^* + \text{H}_2\text{O} + \text{LOO}^*
\end{align*}
\]

In the PLPCOOH reaction, the UVA-dissociated alkoxy radical is intramolecularly rearranged by addition to the \(\alpha,\beta\)-double bond to afford a carbon-centered epoxyalkyl radical, the latter coupling with molecular oxygen to form epoxyperoxyl radicals. \(\alpha\)-Tocopherol can trap peroxy radicals, giving a hydroperoxide and \(\alpha\)-tocopheroxyl radical (\(\alpha\)-T\(^*\)). \(\alpha\)-T\(^*\) could react rapidly and irreversibly with the epoxyperoxyl radicals to form 8α-epoxyPLPC-dioxy-\(\alpha\)-tocopherones (1). On the other hand, PLPC-peroxyl radicals yielded according to Eqs. (2) and/or (3) could react with \(\alpha\)-T\(^*\) to form the corresponding 8α-(PLPC-dioxy)-\(\alpha\)-tocopherones (2).

In conclusion, UVA-induced dissociation of endogenous hydroperoxides is the first step of lipid peroxidation in the present liposomal system. Thus, a trace amount of hydroperoxides in the reaction system might be determinant for UVA-induced lipid peroxidation.

**Acknowledgments**

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**References**