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In Vitro Interaction of Selected Phospholipid Species with Mercuric Chloride Using Fourier Transform ^{1}H-NMR

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Many studies on the mercury toxicities have been accumulated since the outbreak of 'Minamata Disease' (Miura and Imura, 1987). There have been few reports on the reaction mechanisms of mercurials with phospholipids which substantially locate in biological membranes, although the interactions of nucleotides or nucleosides with mercurials have been reported(Mansy et al, 1975, Taylor et al, 1981). Recently, the study on the interaction of mercuric chloride($HgCl_2$) with amino polar heads of model membranes containing phosphatidylserine (PS) and phosphatidylethanolamine(PE) has been reported, as the results from the fluorescence polarization analysis using 1,6-diphenyl-1,3,5-hexatriene (Delnomdedieu et al,1989). We demonstrate here the interactions of dioleoylphosphatidylethanolamine(DOPE) and dioleoylphosphatidylcholine(DOPC) with $HgCl_2$, using fourier transform ^{1}H-NMR(^{1}H-FT-NMR).

MATERIALS AND METHODS

The standard phospholipids,DOPE and DOPC, were obtained from Sigma chemical company(St.Louis, U.S.A.), $CDCl_3$(98 %) was obtained from Aldrich chemical company(Wisconsin, U.S.A.) and D_2O(99.75 %) was purchased from Wako pure chemical industry Co. Ltd.(Osaka, Japan). DOPE or DOPC(2.69 mM)in 1 ml of $CDCl_3$ was reacted with 1 ml of $HgCl_2/D_2O$ solution by shaking for 30 min at room temperature. The $CDCl_3$ layer was concentrated to 600 μ l under N_2 gas. The ^{1}H-FT-NMR was operated at 27 ℃ unless otherwise noted, using a JEOL GSX270(270 mHz, 6.35 T).

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Table 1. Comparison of ^1H chemical shifts(ppm) for functional groups of DOPE and DOPC in the presence of $HgCl_2$

molar ratio	CH_2N	N^+H_3	$N^+(CH_3)_3$	glycerol CH_2OP	lipid-group CH_2OP
$HgCl_2$/DOPE					
saturated[a)]	3.28	8.14		4.00	4.38
1/1	3.20	8.27		3.95	4.36
1/2	3.17	8.28		3.95	4.36
1/10	3.19	8.30		3.95	4.36
control($CDCl_3$)	3.19	8.35 ± 0.035		3.95	4.36
control(D_2O)	3.19	ND[b)]		3.95	4.36
$HgCl_2$/DOPC					
saturated[a)]	3.79		3.23	4.07	4.47
1/1	3.67		3.26	3.93	4.37
control(D_2O)	3.65		3.23	3.92	4.36

a): Supersaturated $HgCl_2$ solution
b): not detected

RESULTS AND DISCUSSION

The chemical shifts for polar headgroups of DOPE and DOPC in the presence of $HgCl_2$ are shown in Table 1. The chemical shift for N^+H_3 resonance of DOPE was 8.35 ± 0.035 ppm before the reaction with $HgCl_2$. The amino peak of DOPE became extremely broad and was difficult to monitor by the reaction with pure D_2O alone. However, for the reaction with $HgCl_2/D_2O$ solution, the N^+H_3 resonance shifted downfield(less than 8.3 ppm) (Fig.1), whereas the N^+H_3 resonance did not shift below 8.3 ppm by the change of the DOPE concentration(in the range of 0.20 mM to 20.2 mM) or the detection temperature(27 ℃ to 42 ℃) of the NMR. Furthermore, a linear correlation between chemical shifts for N^+H_3 resonance and $HgCl_2$ concentrations was shown. On the other hand, the resonance of each functional group of DOPC first shifted upfield when it reacted with supersaturated $HgCl_2$ solution under our experimental conditions. It was suggested that phospholipids bearing amine group on their polar heads were more important target of $HgCl_2$ at least in vitro. In a previous study(Shinada

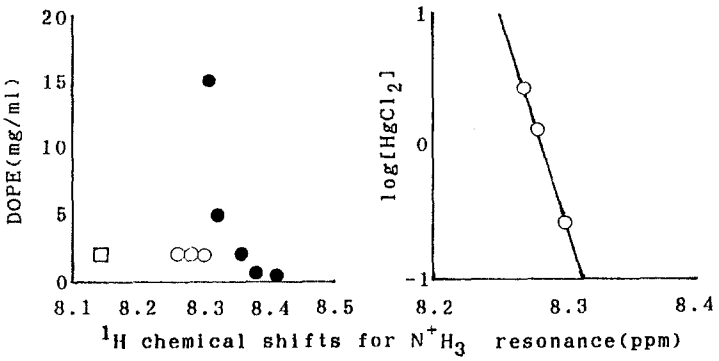

Figure 1. ^{1}H chemical shifts for $N^{+}H_3$ in the presence of $HgCl_2$

○: DOPE in the presence of $HgCl_2$
(molar ratio of $HgCl_2$/DOPE:1/1,1/2,1/10)

□: DOPE in the presence of $HgCl_2$
(supersaturated $HgCl_2$ solution)

●: DOPE in $CDCl_3$ solution

et al, 1990), we reported that phospholipid peroxides, particularly peroxides of PS and PE species bearing amine group on their heads, were induced in rat kidney exposed to $HgCl_2$(0.5 mg/kg/day) for three days(see Table 2), and we suggested that phospholipid peroxidation might be induced by the interactions of amine group of phospholipids with mercurials, although the other mechanisms, such as the mediation of radicals and/or the depression of anti-oxidative enzyme system, have been proposed. Kirschner and Ganther(1982) reported a significant increase of electron density in the lipid headgroup region of the cytoplasmic side of the $HgCl_2$-treated membranes by X-ray diffraction measurement, while PS and PE were mainly located on the cytoplasmic side of membranes(Verkleij et al, 1973, Kleinsmith and Kish, 1988, Sanchez-Yague and Llanillo, 1986 and Pelletier et al, 1988). It was suggested that the reactions of phospholipids bearing amine group on their polar heads with $HgCl_2$ might play an important role in vivo.

Table 2. In vivo induction of phospholipid peroxides (meq./kg) in rat kidney exposed to $HgCl_2$

Period	Group	Phospholipid				
		PI[a]	PS[b]	PE[c]	PC[d]	Total[e]
12hrs[f]	$HgCl_2$	2.57 ± 0.56[g]	4.06 ± 0.81	3.47 ± 0.84	8.82 ± 1.13	6.04 ± 0.79
	Control	1.39 ± 0.51	6.04 ± 1.14	4.85 ± 0.93	8.42 ± 0.77	6.24 ± 0.77
1 day	$HgCl_2$	6.83 ± 2.75	40.2 ± 11.2	15.5 ± 3.96	22.5 ± 3.66	20.5 ± 0.25
	Control	0.89 ± 0.68	7.62 ± 2.13	8.42 ± 0.97	6.24 ± 0.77	6.73 ± 0.08
3 days	$HgCl_2$	2.87 ± 1.56	5.25 ± 0.04	5.74 ± 0.89	4.36 ± 0.08	4.75 ± 0.38
	Control	3.07 ± 2.61	6.34 ± 0.87	6.44 ± 1.72	5.84 ± 1.61	6.24 ± 1.36
5 days	$HgCl_2$	4.25 ± 2.07	8.81 ± 1.05	9.80 ± 2.43	8.32 ± 0.92	8.32 ± 1.27
	Control	3.07 ± 2.39	7.23 ± 2.24	5.84 ± 1.27	7.33 ± 2.13	6.44 ± 1.51

* doses of $HgCl_2$ was 0.5 mg/kg ; 3 organs were analyzed in each group.
a):phosphatidylinositol, b):phosphatidylserine, c):phosphatidylethanolamine, d):phosphatidylcholine, e):Total : $Area(PI+PS+PE+PC)_{235nm}/Area(PI+PS+PE+PC)_{203nm}$, f):time after initial administration, g):mean standard ± deviation

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