Original Article

Partition of 2,4-Dichlorophenoxyacetic Acid Derivatives in Phosphatidylcholine Multi-lamellar Vesicles

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The interaction of 2,4-dichlorophenoxyacetic acid (2,4-D) with multi-lamellar vesicles of dimyristoyl and dipalmitoyl L- α -phosphatidylcholine was examined from the pH dependency of partition and pH- and temperature-dependent polarization of fluorescence probes such as 1,6-diphenyl-1,3,5-hexatriene. It was strongly suggested that 2,4-D in the undissociated form was located at the region from the neighborhood of the glycerol backbone to a shallow site of the hydrophobic core of the lipid bilayers and that its anionic species was adsorbed at the membrane surface. Fluorescence study with the derivatives of 2,4-D showed that hydrophobicity and p*K*_a of these acids determined their interaction with membrane.

Key words: 2,4-dichlorophenoxyacetic acid derivatives, pH dependency of partition in synthetic membranes, polarization of fluorescence probes.

INTRODUCTION

2,4-Dichlorophenoxyacetic acid (2,4-D) and other halogenated phenoxy acids are widely used herbicides and are known to exhibit a hormonal activity at a micromolar concentration.¹⁾ These herbicides also affect both the ultrastructure of cellular membranes and the membrane functions such as permeabilities of ions or small non-electrolytes, oxidative phosphorylation, and electron transport.^{1–3)} Although their mode of action is still obscure, the perturbation on the membrane structure is likely to be one of the main factors to show the biological activity. The permeability and fluidity of the artificial and intact membranes have been examined in the presence of natural plant hormones such as indole-3-acetic acid, abscisic acid, and their analogs.^{3–7)} The undissociated species of these acids were found to be partitioned in membranes, which increased the membrane fluidity. The similar observations have been reported for 2,4-D and its analogs.^{3,4,7–10)}

The p*K*_a values of these acids range from 2.8 to 5.0,^{11–13)} indicating that the corresponding anions are the dominant species under the physiological conditions. Therefore, the structural changes in the membranes caused by the undissociated species could not fully account for the enhanced membrane permeability

towards ions and non-electrolytes. Meanwhile, the NMR studies on the interaction between these acids and lipids have strongly suggested that the anions are adsorbed at the membrane surface consisting of the head group of a lipid.^{14,15)} Furthermore, 2,4-D in the anionic form has been reported to induce the conformational changes of the lipid or protein regions of the plant plasma membrane.¹⁶⁾ The adsorbed anionic species of 2,4-D and auxins are considered to possess a dipolar character and hence, to modify the dipole potential at the membrane/water interface. The carrier-mediated ion transport through membrane has been enhanced by the addition of 2,4-D, suggesting this concept.^{17,18)}

It is very important in understanding the mode of action of these acids to know the site of membrane where the acids are favorably partitioned. The adsorbed state of these acids has been previously examined for the anionic species,^{14,15)} but the site of partition for the undissociated species has been only speculated by the pH dependency of the partition in membranes⁴⁾ and the shift of the thermotropical phase transition temperature.¹⁹⁾ Although the fluorescence studies using probe molecules have been briefly conducted,^{3,10)} their pH dependent profiles useful to estimate the site of partition are not known.

On the basis of these consideration, the pH dependency of partition in multi-lamellar vesicles (MLV) consisting of phosphatidylcholines was first examined for 2,4-D.

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In order to estimate where the anion and undissociated species of 2,4-D were dominantly partitioned, the temperature and pH dependencies of fluorescence profiles of the probe molecules each of which was partitioned in the hydrophobic core (dipenylhexatriene), near the glycerol backbone of membrane (trimethylammonium derivative of diphenylhexatriene), or at the membrane surface (chlorophyll *a*) were examined. The fluorescence intensity and polarization of probe molecules are known to be highly dependent on their molecular motion and microenvironment around them.²⁰⁾ Therefore, the changes of fluorescence profiles caused by pH, temperature and co-existence of herbicides can reveal both microviscosity of membrane and the site of membrane where the herbicides are partitioned. The effect of the structural modification of 2,4-D on the site of partition was also studied by using its structural analogs.

MATERIALS AND METHODS

1. Chemicals

[Carbonyl-¹⁴C]2,4-D (514.3 MBq mmol⁻¹, Fig. 1) was purchased from Sigma (St. Louis). The radiochemical purity was >98%, as determined by radio-HPLC (Waters LC Module I linked with a Radiomatic Flo-one β A-120) equipped with a Sumipax ODS A-212 column (6 mm i.d. $\times$ 15 cm; 0.1% phosphoric acid/acetonitrile=60/40, v/v; flow rate, 2.0 ml min⁻¹). The non-labeled 2,4-D (>99%) from Wako Pure Chemical Co. (Osaka) was used for fluorescence studies without further purification. The other halogenated phenoxy acids (Fig. 1), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T, Tokyo Kasei), 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB, Aldrich) and 2-(2,4-dichlorophenoxy)propionic acid (dichlorprop, Aldrich) were purified by HPLC as the chemical purity became >99%.

L- α -Phosphatidylcholines (>99%), dimyristoyl (DMPC) and dipalmitoyl (DPPC), and that of egg yolk (type XVI-E, EggPC) from Sigma, stearylamine (>99%, SA) and dicetyl phosphate (>99%, DCP) from Aldrich (Milwaukee) were obtained. 1,6-Diphenyl-1,3,5-hexatriene (DPH) and 1-(4-trimethylammonium) phenyl-6-phenyl-1,3,5-hexatriene iodide (tma-DPH) from Wako

Pure Chemical Co. and chlorophyll *a* (*Chla*, from spinach, substantially free from chlorophyll *b*) from Sigma were used as a fluorescence probe without further purification. Pure water with an electrical conductivity of $5.5 \times 10^{-8} \Omega^{-1} \text{cm}^{-1}$ supplied from a Puric Model R (Organo Co., Ltd., Osaka) was used to prepare buffer solutions.

2. pH Dependency of Partition of 2,4-D in MLV

The 1 mM MLV dispersion of DPPC, DMPC or EggPC was prepared in 10 mM acetate (pH 2.3, 3.0 and 3.5) or phosphate (pH 7.5) buffer containing 0.1 M KCl and subjected to the partition study as reported previously.²⁰⁾ Methanol solution (10 μ l) of [¹⁴C]2,4-D was well mixed with a 1-ml aliquot of the MLV dispersion at room temperature and immediately incubated in darkness at 25°C for 4 hr. The final concentration of 2,4-D was about 1.6 ppm. The partition coefficient ($K_{L/W}$) with a trapping efficiency of lipid at the glass fiber filter (f) used to separate MLV from buffers were estimated in the same manner as reported previously.²⁰⁾ Furthermore, the MLV consisting of DPPC (or DMPC) and SA (or DCP) at the molar ratio of 10:1 was similarly prepared for the partition study to examine if the charge at the membrane surface affected the partition of 2,4-D. The experiments were conducted at pH 3.0 and 7.5 where SA and DCP were positively and negatively charged, respectively. All experiments were repeated five times.

3. Fluorescence Study

The fluorescence probes (DPH and tma-DPH) were utilized to estimate the location of 2,4-D and its related three acids in the membrane. The MLV dispersion containing the acid, probe and lipids was similarly prepared at pH 3.0 and 7.5. The final lipid concentration was 0.1 mM. The molar ratio of the probe to lipid was 2×10^{-3} and that of 2,4-D to lipid (*R*) was changed from 0.1 to 1.5. The MLV containing 2,4,5-T, 2,4-DB and dichlorprop (*R*=1.0) was prepared for the fluorescence polarization study to examine the effect of chemical structure as well as hydrophobicity and *pK_a*. The fluorescence polarization of probes (DPH and tma-DPH) at 430 nm (*P*) was obtained in the same manner as reported previously.²⁰⁾ The temperature dependency of polarization was examined at the ranges from 14 to 30°C and 25 to 50°C for DMPC- and DPPC-MLV's, respectively, to know the effect of 2,4-D on the membrane fluidity. The experiment was conducted in triplicate.

Chla was used as a another fluorescence probe to monitor the pre-transition of the DPPC-MLV at ca. 23°C,²¹⁾ since its porphyrin moiety was known to be located at the membrane surface. The molar ratio of *Chla* to DPPC was 2×10^{-3} and the MLV was prepared at pH 7.5 similarly as above but under N₂ atmosphere in darkness to prevent oxidation of *Chla*. The excitation wave-

Fig. 1 Chemical structures of the halogenated phenoxy acids used in the partition and fluorescence polarization studies.

length was 420 nm and the fluorescence intensity at 670 nm was monitored at the temperature range from 16 to 44°C. The effect of 2,4-D on the pre-transition was examined by changing R from 0.01 to 1.5. The similar experiments were also conducted by using 2,4,5-T, 2,4-DB and dichlorprop at $R=1.0$.

RESULTS

1. Partition of 2,4-D in MLV

The trapping efficiency of lipid (f) was $>98\%$ for the MLV tested except that from EggPC ($87.5 \pm 1.5\%$). By using these values, the partition coefficients ($K_{L/W}$) of 2, 4-D were calculated, as listed in Table 1. The $K_{L/W}$ value showed maximum at pH 3.0 under the pH range tested and this profile was common to all MLV's. The order of the $K_{L/W}$ value in MLV was DMPC $>$ EggPC $>$ DPPC at every pH tested. The incorporation of SA or DCP (mixed MLV) reduced the partition but did not significantly change this order. Since the headgroup of DMPC and DPPC was amphiphilic at the pH range from 2.5 to 11.5,²²⁾ SA and DCP were considered to add the positive and negative charges at the membrane surface, respectively.

2. Fluorescence Polarization of DPH and tma-DPH in MLV

In the absence of 2,4-D, the fluorescence polarization

of DPH and tma-DPH in the gel phase (P_G) of DPPC-MLV at 24–34°C exhibited almost the constant value, as listed in Table 2 ($R=0.0$). The slightly larger P_G values were obtained at pH 3.0 than 7.5. As with an increase of temperature, the polarization gradually decreased and dramatic changes were detected at 42.2°C (pH 7.5) and 44.6–44.8°C (pH 3.0). The former value was close to the thermotropic phase transition temperature (T_c) reported previously.²³⁾ These differences in polarization and T_c implied that microviscosity of the membrane was higher at pH 3.0 than 7.5. The incorporation of 2,4-D into DPPC-MLV at pH 3.0 reduced the P_G value for both probes in concomitant with the lower-temperature shift of T_c . The P_G value decreased from 0.403 ± 0.001 ($R=0.1$) to 0.390 ± 0.003 ($R=1.5$) for DPH and the larger change was detected for tma-DPH (0.400 ± 0.001 , $R=0.1$; 0.367 ± 0.007 , $R=1.5$). The temperature range of the phase transition was broadened at $R=1.5$ and T_c decreased by 1.8–2.0°C, as shown in Figs. 2 and 3. In contrast, 2,4-D was found to cause the insignificant change in polarization in the liquid phase (P_{LC}) at pH 3.0, and the polarization profiles for both probes at pH 7.5 were not significantly affected even at the high concentration of 2,4-D ($R=1.5$).

Similar results were obtained for DMPC-MLV, as listed in Table 3. The T_c values at pH 3.0 and 7.5 were estimated to be 27.2–27.4°C and 25.5°C, respectively, and

Table 1 pH Dependency of partition coefficient of 2,4-D in the various MLV's.

MLV	$K_{L/W}$ at 25°C			
	pH 2.3	pH 3.0	pH 3.5	pH 7.5
EggPC	107.4 ± 6.5	206.4 ± 13.2	108.9 ± 11.7	83.6 ± 13.2
DMPC	170.5 ± 12.5	592.1 ± 71.6	385.6 ± 23.4	105.7 ± 5.4
DMPC+SA ^a	– ^b	208.1 ± 3.8	–	78.1 ± 10.6
DMPC+DCP ^a	–	220.2 ± 32.2	–	61.4 ± 5.4
DPPC	98.0 ± 10.1	113.3 ± 5.5	98.8 ± 5.4	74.0 ± 8.7
DPPC+SA	–	86.3 ± 5.7	–	64.1 ± 11.7
DPPC+DCP	–	99.0 ± 4.9	–	66.1 ± 8.9

^a) Molar ratio to lipid was 0.1. ^b) Not determined.

Table 2 Effect of 2,4-D on the fluorescence polarization and phase transition temperature in DPPC-MLV.

pH	R^b	DPH ^a			tma-DPH ^a		
		P_G^c	P_{LC}^d	T_c^e	P_G	P_{LC}	T_c
3.0	0.0	0.402 ± 0.003	0.155 ± 0.021	44.6	0.403 ± 0.001	0.266 ± 0.003	44.8
	0.1	0.403 ± 0.001	0.155 ± 0.012	44.7	0.400 ± 0.001	0.263 ± 0.013	43.8
	0.5	0.393 ± 0.005	0.154 ± 0.026	44.6	0.393 ± 0.001	0.268 ± 0.006	43.5
	1.0	0.392 ± 0.006	0.158 ± 0.021	44.4	0.379 ± 0.005	0.259 ± 0.004	43.1
	1.5	0.390 ± 0.003	0.152 ± 0.016	43.8	0.367 ± 0.007	0.254 ± 0.007	42.8
7.5	0.0	0.391 ± 0.002	0.153 ± 0.017	42.2	0.379 ± 0.003	0.255 ± 0.004	42.2
	1.5	0.390 ± 0.002	0.145 ± 0.007	42.2	0.377 ± 0.004	0.251 ± 0.006	42.2

^a) Molar ratio to DPPC (0.1 mM), 2.0×10^{-3} . ^b) Molar ratio of 2,4-D to DPPC. ^c) Fluorescence polarization at 430 nm in the gel phase (at 24–34°C). ^d) Fluorescence polarization at 430 nm in the liquid crystalline phase (at 46–50°C). ^e) Phase transition temperature, in degrees.

the latter was in agreement with the reported T_c value.²³⁾ A similar effect by 2,4-D on the pH and temperature dependencies of polarization was observed. As compar-

Fig. 2 Effect of 2,4-D and its derivatives on the temperature dependency of the fluorescence polarization due to DPH in DPPC-MLV at pH 3.0. Molar ratio of herbicides to DPPC (R) was 1.0.

Fig. 3 Effect of 2,4-D and its derivatives on the temperature dependency of the fluorescence polarization due to tma-DPH in DPPC-MLV at pH 3.0. Molar ratio of herbicides to DPPC (R) was 1.0.

ed with DPPC-MLV, the larger shift of T_c was detected for DMPC-MLV. The shifts to the lower temperature were 3.6°C and 4.7°C for DPH and tma-DPH at $R=1.5$, respectively.

When the other halogenated phenoxy acids, 2,4,5-T, 2,4-DB and dichlorprop, were added as a constituent of DPPC-MLV at pH 3.0, the temperature dependency of the fluorescence polarization for DPH and tma-DPH was greatly changed depending on their chemical structures (Figs. 2 and 3). The order of the perturbation on DPPC-MLV was 2,4-DB > 2,4,5-T > dichlorprop > 2,4-D for both probes. In the case of DPH/DPPC-MLV, 2,4-D, 2,4,5-T and dichlorprop caused the smaller change in the P_G value of DPH, but 2,4-DB reduced it by about 0.02–0.04 together with the dramatic broadening of the phase transition temperature range. The T_c values were graphically estimated to be 44.4°C (2,4-D), 40.7°C (2,4,5-T), 37.4°C (2,4-DB) and 42.6°C (dichlorprop). The P_{LC} value was not so largely affected by addition of these herbicides. As compared with DPH, the fluorescence polarization of tma-DPH was slightly more reduced at the temperature range tested by addition of these herbicides. The T_c values were estimated to be 43.1°C (2,4-D), 40.3°C (2,4,5-T), 37.2°C (2,4-DB) and 42.2°C (dichlorprop), showing that the T_c values estimated by the two fluorescence probes were almost the same. At pH 7.5, there was no significant change in the fluorescence polarization for both probes even when these herbicides were added at the molar ratio of 1.5 to DPPC.

3. Effect on the Pre-transition of DPPC-MLV

The pre-transition temperature (T_c') of the DPPC-MLV was graphically estimated by the temperature dependency of the fluorescence intensity at 670 nm due to *Chla*. In the absence of the herbicides, T_c' was determined to be 31.5°C at pH 7.5 which was in the range of the reported value (29–34°C).²¹⁾ As with an increase of the concentration of 2,4-D, the T_c' value gradually shifted finally to 27.5°C ($R=1.5$), as listed in Table 4. The

Table 3 Effect of 2,4-D on the fluorescence polarization and phase transition temperature in DMPC-MLV.

pH	R^b	DPH ^a			tma-DPH ^a		
		P_G^c	P_{LC}^d	T_c^e	P_G	P_{LC}	T_c
3.0	0.0	0.387±0.005	0.169±0.017	27.2	0.389±0.003	0.256±0.003	27.4
	0.1	0.388±0.004	0.170±0.012	26.4	0.390±0.002	0.266±0.010	27.2
	0.5	0.389±0.004	0.169±0.012	25.5	0.377±0.003	0.258±0.004	25.5
	1.0	0.373±0.007	0.169±0.013	24.4	0.366±0.003	0.262±0.007	24.3
	1.5	0.375±0.009	0.173±0.018	23.6	0.357±0.008	0.261±0.006	22.7
7.5	0.0	0.383±0.002	0.178±0.008	25.5	0.383±0.001	0.260±0.006	25.5
	1.5	0.383±0.005	0.174±0.017	25.6	0.382±0.005	0.263±0.003	25.6

^{a)} Molar ratio to DMPC (0.1 mM), 2.0×10^{-3} . ^{b)} Molar ratio of 2,4-D to DMPC. ^{c)} Fluorescence polarization at 430 nm in the gel phase (at 14–20°C). ^{d)} Fluorescence polarization at 430 nm in the liquid crystalline phase (at 29–32°C). ^{e)} Phase transition temperature, in degrees.

Table 4 Effect of herbicides on the pre-transition temperature of DPPC-MLV.

Compd.	Conc. ^{a)}	T _c ' ^{b)}
2,4-D	0.0	31.5
	0.01	31.0
	0.02	30.5
	0.1	29.0
	1.0	28.5
	1.5	27.5
2,4,5-T	1.0	29.8
2,4-DB	1.0	25.0
Dichlorprop	1.0	28.8

^{a)} Molar ratio of herbicides to DPPC (0.1 mM). ^{b)} At pH 7.5, in degrees.

effect of 2,4,5-T and dichlorprop on T_c' was almost the same as 2,4-D at the molar ratio of 1.0 to DPPC, while the addition of 2,4-DB caused a larger shift of T_c' to a lower temperature by 6.5°C (Table 4). Since the pre-transition corresponds to the structural change at the membrane surface,²¹⁾ these results show that the herbicides in an anionic form would alter the configuration of the head group of the lipid.

DISCUSSION

The lipid-soluble molecules are known to affect the membrane structure and the site of their partition in membranes has been discussed based on the phase transition profiles examined by differential scanning calorimetry²⁴⁾ and various spectroscopic methods.^{10,15,25)} It is generally considered that the hydrophobic non-electrolytes are solubilized in the acyl chain region of lipid bilayers but that the polar molecules are partitioned in the vicinity of a lipid glycerol backbone. The ionic species are most likely to be adsorbed at the membrane surface via coulombic interaction with the phosphatidylcholine head group.²⁴⁾ The pK_a value of 2,4-D was about 2.9^{11,12)} and hence, the percentage of the undissociated species present in each buffer could be calculated to be 79.9% (pH 2.3), 44.3% (pH 3.0), 20.1% (pH 3.5) and 0% (pH 7.5). The larger partition of the undissociated species in MLV was expected for 2,4-D under more acidic conditions, but the smaller K_{L/W} value was obtained at pH 2.3 than 3.0. The phosphate moiety of the lipid head group is considered to be mostly protonated below pH 2.5, which resulted in the closer packing of the acyl chains with the concomitant increase of T_c.²²⁾ The change in the packing of lipids could be monitored by the extent of fluorescence polarization in the gel phase.^{15,16)} The larger P_G values together with the higher T_c under the acidic conditions than those at pH 7.5 (Tables 2 and 3) showed the closer packing which played a role in squeezing 2,4-D from the membranes. Therefore, the balance between the extent of dissociation and the lipid

packing resulted in the pH dependency of K_{L/W}.

In the case of the mixed MLV's, the addition of a charge at the membrane surface caused the similar effect as protonation, which led to the closer lipid packing and as a result, the reduction of K_{L/W} might occur. The slightly larger K_{L/W} value in the positively charged mixed MLV of DMPC than the negatively charged one at pH 7.5 seems to imply the contribution of coulombic interaction, while the opposite trend on K_{L/W} was observed at pH 3.0 in the mixed MLV's of DPPC and DMPC (Table 1). Since it is known that the pH very close to a negatively charged membrane surface can be substantially lower than in the bulk phase,^{4,13)} less dissociation than estimated by a bulk pH would result in more partition of 2,4-D into MLV by incorporation of DCP.

Meanwhile, the T_c values of DPPC, DMPC and EggPC were 41°C, 23°C and -15~-17°C, respectively.²³⁾ It has been reported that the partition of a chemical in the hydrophobic region of the lipid acyl chains is most facilitated at the neighborhood of T_c.²⁵⁾ Therefore, the largest K_{L/W} value at 25°C would be obtained for the DMPC-MLV. The ratios of K_{L/W} to DPPC-MLV at pH 7.5 were 1.13 and 1.43 for EggPC- and DMPC-MLV's, respectively, and increased to 1.82 and 5.23 at pH 3.0. Since about 50% and 0% of 2,4-D were present in the undissociated form at pH 3.0 and 7.5, respectively, these results strongly suggested that the undissociated 2,4-D was partitioned in the interior of the lipid bilayers of MLV and that the anionic 2,4-D was simply adsorbed at the membrane surface probably by putting the phenyl moiety in the head group region of lipids.

The site in the membrane where 2,4-D was partitioned could be also estimated by the fluorescence polarization profiles. DPH is a hydrophobic fluorescence probe and is most likely to be present in the hydrophobic core of the membrane, the center of the lipid bilayer.²⁶⁾ In contrast, tma-DPH possesses the hydrophilic moiety at one of the molecular ends and is considered to be mainly partitioned at the region from the membrane surface to the vicinity of the glycerol backbone.²⁷⁾ The existence of a chemical at the same region where the fluorescence probe is partitioned would decrease the microviscosity of the region. The molecular motion of the probe is facilitated, which leads to the reduction of fluorescence polarization. The decrease of P_G by addition of 2,4-D was smaller for DPH than tma-DPH in DMPC- and DPPC-MLV's at pH 3.0, indicating that the undissociated 2,4-D was most likely partitioned in the vicinity of tma-DPH rather than DPH. Meanwhile, the lower-temperature shift of T_c means the disintegration of the close packing of the acyl chains. The shift was only about 2°C in a maximum for DPPC-MLV but was enhanced to 3.6-4.7°C for the DMPC-MLV. The larger membrane fluidity in the gel phase was expected for the DMPC-MLV due to

the shorter acyl chain²³⁾ and hence, the partition of the undissociated 2,4-D into the hydrophobic core would be facilitated, which resulted in the larger shift of Tc.

If the undissociated species of a chemical is partitioned in the interior of the bilayer, its extent would be dependent on the pK_a and hydrophobicity. The log P values of the herbicides representing their hydrophobicity have been reported to be 2.83 (2,4-D), 3.31 (2,4,5-T), 3.53 (2,4-DB) and 3.43 (dichlorprop).¹²⁾ Furthermore, the percentage of the undissociated form at pH 3.0 could be calculated based on their pK_a values (2.90, 2,4-D; 2.83, 2,4,5-T; 4.95, 2,4-DB; 2.86, dichlorprop)^{11,12)} to be 40–44% for 2,4-D, 2,4,5-T and dichlorprop, and 98.9% for 2,4-DB. The relative order in the partition would be dichlorprop > 2,4,5-T > 2,4-D based on their log P values, since the similar amounts of the undissociated species are expected at pH 3.0. More partition of a chemical causes more decrease of P_G together with the lower-temperature shift of Tc. The same order of these values has been reported for the small unilamellar vesicles of DPPC at pH 3.0,¹⁹⁾ but the corresponding order estimated by the reduced extent of fluorescence polarization in this study was 2,4,5-T > dichlorprop > 2,4-D. The reason of disagreement between the two studies was not clear but our result was in agreement with the previous observation for the branched solute molecule being partitioned in the MLV.²⁸⁾ The branching seemed to be discriminated by the ordering of the acyl chains in MLV and hence, the lower partition was obtained for the branched solute than the straight-chain one. In the case of 2,4-DB, most of the solute was considered to have the undissociated form at pH 3.0. Therefore, the higher partition would cause the larger change in the fluorescence polarization profiles than 2,4-D, 2,4,5-T and dichlorprop (Figs. 2 and 3). These results together with the larger decrease of P_G in tma-DPH than in DPH again suggested that the halogenated phenoxy acids were partitioned in the vicinity of tma-DPH. This most probable site for the partition of the undissociated species, the hydrophilic region near the glycerol backbone or the neighborhood of the ester moieties, has been previously suggested for auxins,^{7,8)} atrazine²⁹⁾ and phenylamide herbicides including carbamates and ureas³⁰⁾ whose hydrophobicity were similar to those of the herbicides tested in this study.

The adsorption of the anionic species of the herbicides tested at pH 7.5 was most likely based on the previous works on partition of hydrophilic and ionic compounds²⁴⁾ and NMR studies on the interaction between lipids and auxins.^{14,15)} The pre-transition is known to correspond to the structural change of lipids at the membrane surface.²¹⁾ Therefore, the lower-temperature shift of Tc' with the increase of 2,4-D added indicated that the anionic species of 2,4-D was located at the membrane surface consisting of the phosphatidylcholine moieties. 2,4,5-T and dichlorprop exhibited the

similar shift of Tc', while the larger shift was detected for 2,4-DB. Since 2,4-DB possessed the longest straight chain structure than others (Fig. 1), the molecule other than the carboxyl group would be anchored into the membrane most favorably. This difference was likely to result in the more perturbation on the lipid packing at the membrane surface.

The membrane constituents of plant cell are very complex and hence, its microenvironment is considered very different from that of MLV consisting of simple lipids. However, the basic profiles of the tested herbicides in this study can be applied to speculate their effects on intact plant cellular membranes. These herbicides most likely exist in the dissociated form and would interact with membrane mostly in the gel phase at neutral pH and around room temperature. In conclusion, both the insignificant change of fluorescence polarization of the two probes at pH 7.5 in the gel phase and the significant effect on the pretransition of membranes imply that these herbicides are predominantly partitioned around the area from the lipid head group to glycerol backbone under the physiological conditions. Although the extent of perturbation on membrane fluidity is considered to depend on the chemical structure of phenoxyalkanoic acids, this modification would most probably alter the membrane ultrastructure and functions such as ion permeability or oxidative phosphorylation, which closely relates to the herbicidal action.²⁾

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要 約

2,4-D 誘導体のホスファチジルコリン多重層リポソームへの分配

片木敏行

ホスファチジルコリン多重層リポソーム中の蛍光プローブに由来する蛍光偏光, 蛍光強度の pH, 温度依存性並びに膜の相転移温度に与える 2,4-D 誘導体の影響を調べた. pH 3.0 で非解離型の 2,4-D は膜ゲル状態における蛍光偏光の減少とともに膜の相転移温度を低下させ, pH 7.5 での解離型は膜表面における前転移温度を低下させた. 蛍光実験並びに多重層リポソームへの分配係数の pH 依存性から 2,4-D 誘導体の非解離型は脂質二重膜内部のグリセロール骨格付近から疎水性領域にかけて分配され, その程度は疎水性, 酸解離定数, 化学構造に左右された. 一方, 解離型は膜表面に吸着するものと推定された.