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Distribution of tumor promoters in lipid membranes and changes in membrane structure

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The interaction of tumor promoters differing in molecular structure, namely, 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and teleocidin, with dipalmitoylphosphatidylcholine (DPPC) vesicles was studied. Investigation by Fourier transform infrared spectroscopy clarified the differences between the tumor promoters in the mode of interaction with lipid bilayer membranes. The temperature dependence of the bandwidth of the C–H or C=O stretching absorption of lipid molecules in the presence of tumor promoters relative to that in pure DPPC vesicles indicated that TPA is incorporated into the hydrophobic core of the lipid bilayer membrane whilst teleocidin binds predominantly to the membrane surface. However, both tumor promoters tend to restrict the motion of lipid molecules in membranes. The same conclusion was derived from measurements of steady-state fluorescence polarization, which showed that tumor promoters decreased the membrane fluidity. On the other hand, carboxyfluorescein (CF) leakage from vesicles was enhanced by the addition of TPA below the phase-transition temperature, whereas the effect of teleocidin on steady-state CF leakage was not as significant. It is considered that the difference in the profile of the TPA-induced increase in CF leakage compared to that of teleocidin might be ascribable to a different binding site for each tumor promoter in the membranes.

1. Introduction

The two-stage concept of chemical carcinogenesis involves initiation and promotion processes [1]. Various kinds of chemical substances participating in the latter process have been discovered [2–4] and classified into two groups, TPA (12-*O*-tetradecanoylphorbol 13-acetate)- and non-TPA-type promoters. TPA, teleocidin, and aplysiatoxin (fig. 1) are known to be TPA-type tumor promoters, since they possess similar biological activities [5–7], and bind to the same membrane protein in the cell [7,8]. These tumor promoters have also been shown to activate protein kinase C in vitro [9–13].

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However, the question as to whether TPA signaling to cells is mediated by events occurring in the membrane or by binding to specific receptor proteins in the cytosol remains subject to controversy. Liskamp et al. [14] and Tran and Deugnier [15] have shown that fluorescein-conjugated TPA rapidly entered cells and bound to a specific site, supposedly like protein kinase C in the cytosol. On the other hand, others have reported finding receptors for phorbol esters in membrane fractions [16–18]. In addition, it was recently found that phosphorylation of histone, myosin light chain, and troponin I by protein kinase C required a large excess of phospholipid, Ca^{2+}, and diacylglycerol (or phorbol ester). Furthermore, the phorbol ester is supposed to accelerate the binding of protein kinase C to mem-

branes by perturbing the conformation of the enzyme [19–21]. Also, Ashendel [13] has proposed that protein kinase C is activated by binding to lipid bilayer membranes and that phorbol esters take part in the regulation of the conformation of protein kinase C in membranes. In contrast, diacylglycerol (phorbol ester) has been shown to promote aggregation of phosphatidylserine in membranes to yield a hydrophobic region at the membrane surface [22,23]. It has also been supposed that protein kinase C binds to such a hydrophobic region. Therefore, it is of great importance to study the interaction of tumor promoters with lipid bilayer membranes and changes in the membrane structure, having been frequently considered to be responsible for the activity of membrane-associated proteins [24].

Since molecules of TPA and teleocidin exhibit hydrophobicity, they should be distributed to lipid membrane. Indeed, phorbol esters have been reported to interact primarily with phospholipids, not with proteins [25,26]. Malaisse and Deleers [27] have reported that membrane properties were changed by the distribution of TPA to membrane. Here, the binding site of tumor promoters in lipid bilayer membrane was studied by Fourier transform infrared spectroscopy. In addition, the influences of tumor promoters on the membrane fluidity and disturbance in membrane structure were investigated by using fluorescence spectroscopic methods.

2. Materials and methods

2.1. Chemicals

Dipalmitoylphosphatidylcholine (DPPC) was purchased from Sigma. Carboxyfluorescein (CF) from Eastman Kodak was purified by column chromatography [28]. TPA and phorbol were obtained from LC Services Corp. The preparations of teleocidin, aplysiatoxin, and debromoaplysiatoxin have been described previously [29,30]. The highest grade of reagents available was used.

2.2. Fourier transform infrared (FT-IR) spectroscopy

Samples for FT-IR spectroscopy were examined in a CaF_2 cell (50 μm optical path length)

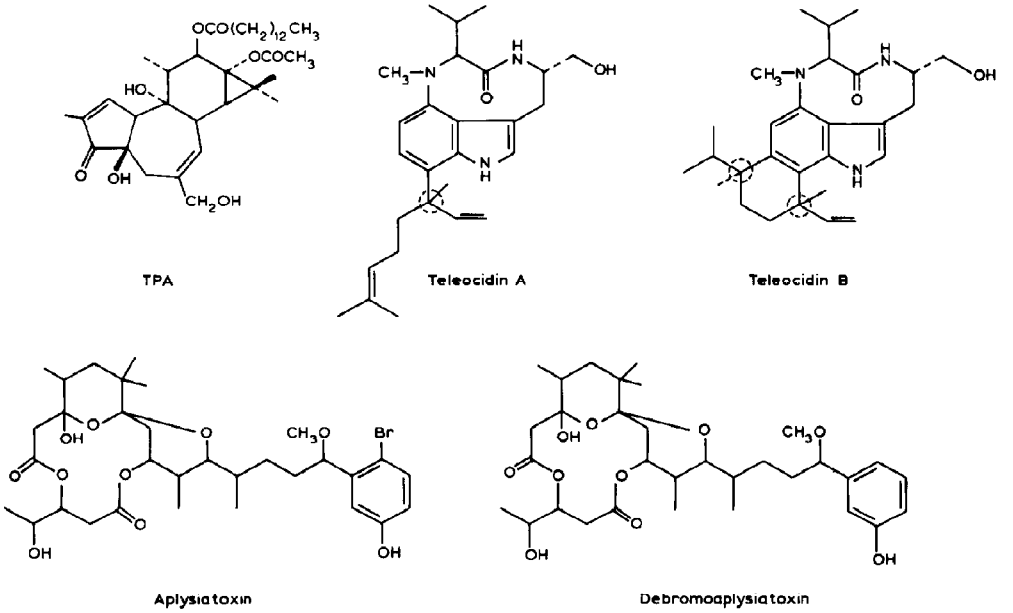

Fig. 1. Structural formulae of the tumor promoters.

equipped with a thermostatted water jacket for temperature control. Measurements were carried out on a Digilab FTS-15E. 200 interferograms were collected with a resolution of 4 cm^{-1}, and the data obtained were encoded every 2 cm^{-1}. Multilamellar vesicles (MLV) were used for measurements. To DPPC (5 mg) in $CHCl_3$ solution was added TPA or teleocidin in ethanol at a lipid/additive molar ratio of 50. The solvent was evaporated under N_2, and the residual film on the glass wall was completely dried under reduced pressure. ^2H_2O-containing buffer (100 mM NaCl, 10 mM Hepes; pH 7.4, uncorrected; 0.5 ml) was added and agitated by using a vortex mixer above the phase transition temperature of the membrane. Heating and cooling processes across the phase transition temperature were repeated several times to obtain stable MLV. The difference infrared spectra of DPPC MLV were recorded in the regions of the C–H and C=O stretching frequencies after subtraction of the standard ^2H_2O buffer spectrum.

2.3. Measurements of membrane fluidity

Single unilamellar vesicles (SUV) were prepared by sonication of a lipid dispersion in buffer solution (100 mM NaCl, 10 mM Hepes, pH 7.2), followed by ultracentrifugation at $100000 \times g$ to remove the Ti particles and larger vesicles. The steady-state fluorescence polarization of 1-anilinonaphthalene-8-sulfonate (ANS) and 1,6-diphenyl-1,3,5-hexatriene (DPH) was measured in order to evaluate membrane fluidity at the membrane surface and the hydrophobic core of the lipid bilayer membrane, respectively. Excitation and monitoring wavelengths were 398 and 480 nm for ANS, and 357 and 450 nm for DPH, respectively. Fluorescence measurements were carried out on an MPF-4 spectrophotometer (Hitachi, Japan). For polarization measurements, special equipment was installed, in which the polarizer on the monitor side was fixed, that on the excitation side being rotated by a stepping motor to 0 or 90° with respect to the other polarizer. At each position, four data points were processed by a microcomputer to be averaged. One averaged polarization value was obtained every 30 s. Membrane fluidity was estimated on the basis of the reciprocal of polarization ($1/P$).

CF leakage from CF-containing vesicles was measured according to the method reported by Barbet et al. [31]. Excitation and monitoring wavelengths were 470 and 520 nm, respectively. These values were chosen in order to reduce as effectively as possible the effect of light scattering. All measurements were carried out within 1 day subsequent to vesicle preparation. Lipid concentration was quantitated according to the method of Raheja et al. [32].

3. Results and discussion

3.1. FT-IR spectroscopy

The frequency and bandwidth of C–H symmetric and C=O stretching absorptions of DPPC vesicles were measured at various temperatures. The temperature dependence of the frequency of C–H symmetric absorption was not markedly affected by the addition of tumor promoters, whereas a significant change in bandwidth was observed.

Fig. 2 shows the effect of TPA on the temperature dependence of the bandwidth of the C–H stretching absorption. Below the phase transition temperature of the membranes, the bandwidth in the presence of TPA is similar to that in its absence; however, above the phase transition temperature, a significant decrease occurred in the presence of TPA. The decrease in bandwidth signifies that the librational and torsional motions of lipid molecules in the membrane are restricted [33]. On the other hand, the temperature dependence of the bandwidth of C=O stretching in the presence of TPA was not significantly altered with respect to that of pure DPPC vesicles. These results indicate that TPA should be distributed mainly in the hydrophobic core of lipid bilayer membranes.

The effect of teleocidin on bandwidth was greater than that of TPA. As shown in fig. 3, the bandwidth of the C–H stretching absorption was reduced throughout the entire range of temperature in the presence of teleocidin. Furthermore, the bandwidth of the C=O stretching absorption

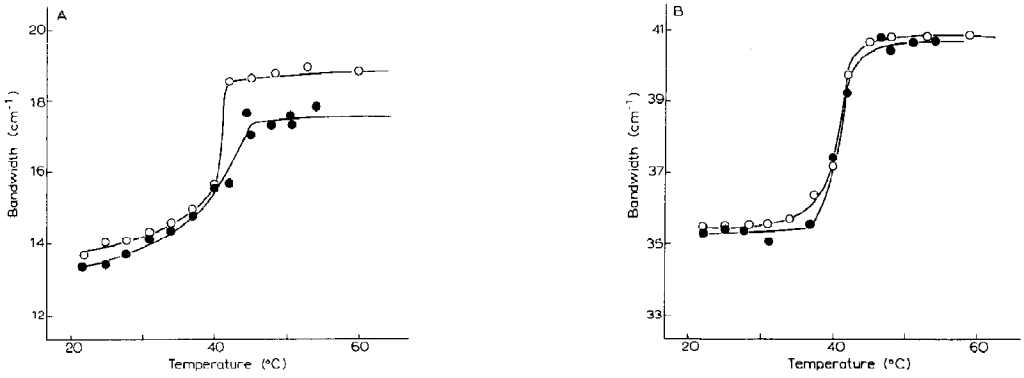

Fig. 2. Temperature dependence of bandwidths of C–H symmetric (A) and C=O (B) stretching absorptions of DPPC vesicles in the absence (○) and presence (●) of TPA ([TPA]/[DPPC] = 1 : 50).

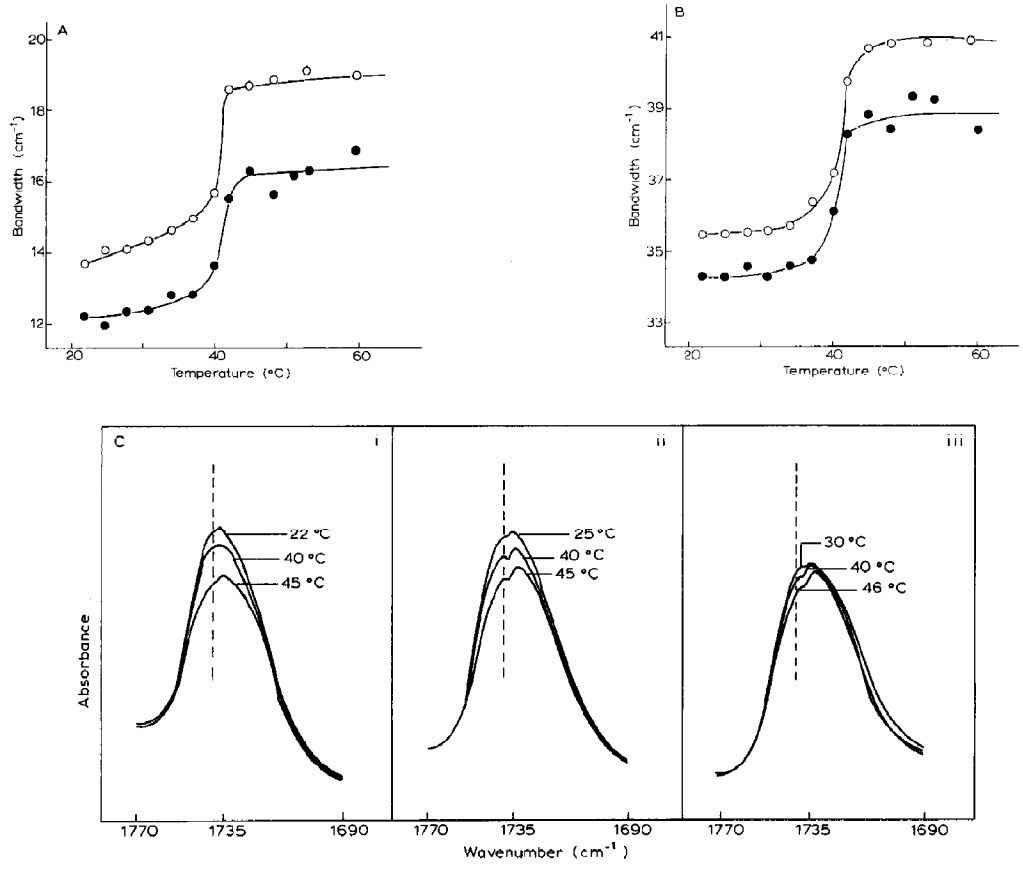

Fig. 3. Temperature dependence of bandwidths of C–H symmetric (A) and C=O (B) stretching absorptions of DPPC vesicles in the absence (○) and presence (●) of teleocidin ([teleocidin]/[DPPC] = 1 : 50). (C) FT-IR spectra of C=O stretching region of: (i) pure DPPC vesicles, (ii) plus TPA ([TPA]/[DPPC] = 1 : 50) and (iii) plus teleocidin ([teleocidin]/[DPPC] = 1 : 50) as a function of temperature (values in ° C listed beside the curves).

also diminished on addition of teleocidin to membranes; indicating that it binds to the membrane surface. This might indicate that the freedom of motion of the carbonyl groups as well as alkyl chains is restricted in DPPC vesicles containing teleocidin relative to that in pure DPPC vesicles [34]. However, it should be noted that the C=O stretching absorption band comprises two components, assigned to the *sn*-1 and *sn*-2 ester carbonyl groups of the glycerol structure [34]. Analysis of Fourier transform deconvolution for resolution enhancement of the C=O stretching absorption yielded curves showing two or three components to exist in the spectra, thus rendering the quantitative interpretation of change in frequency of each component with alteration in temperature inaccurate. However, it appears from the spectra that the component at lower frequency shifts to higher wave numbers with falling temperature (fig. 3C). Therefore, teleocidin is considered to induce a conformational change in the lipid head groups. Taken together, the data indicate that teleocidin binds to the surface region of lipid bilayer membranes, and may promote dehydration of the head groups of lipid molecules through binding to this region, thereby restricting the motional freedom of lipids in the membrane [35]. This molecular mechanism is similar to that proposed by Jacobs and White [36].

In contrast to the results with TPA and teleocidin, phorbol, which lacks the long acyl chain of TPA and does not display tumor-promoting activity, had no effect on the temperature dependence of the FT-IR spectra of lipid bilayer membranes (fig. 4).

3.2. Effect of tumor promoters on membrane fluidity

TPA and teleocidin were shown by FT-IR measurements to be distributed to different sites on the lipid bilayer membrane. However, they both tend to restrict the motion of the lipid molecules in a membrane. This effect exerted by tumor promoters on the properties of membranes was also confirmed in determinations of membrane fluidity. Membrane fluidity was monitored by employing the steady-state fluorescence polarization technique.

DPPC SUV were chosen as the model membrane. Typical experimental results with DPPC vesicles are shown in fig. 5, the data being summarized in table 1. Phorbol had no effect on membrane fluidity under all experimental conditions employed. On the other hand, teleocidin clearly decreased membrane fluidity at the membrane surface (monitored using ANS) as well as at the hydrophobic core of the lipid bilayer membranes (monitored with DPH) over the entire tem-

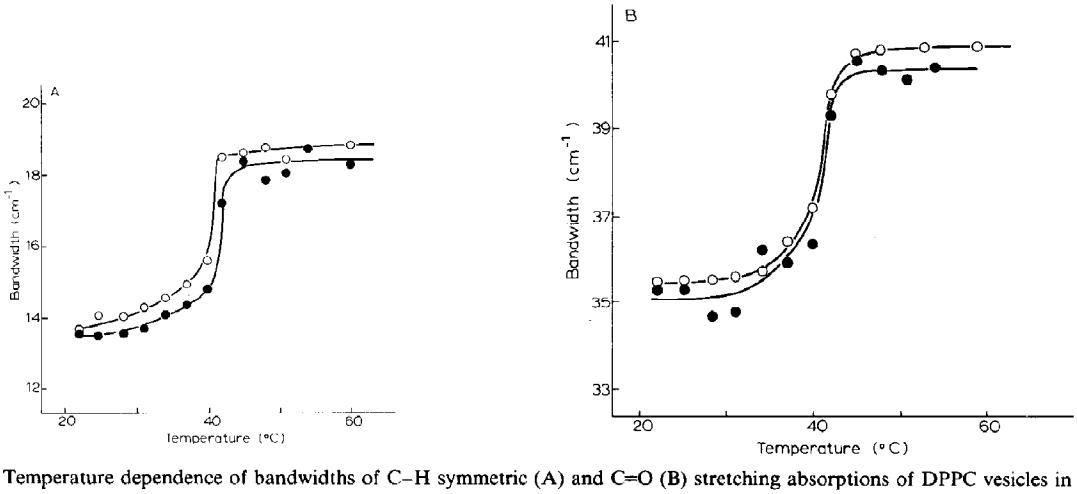

Fig. 4. Temperature dependence of bandwidths of C–H symmetric (A) and C=O (B) stretching absorptions of DPPC vesicles in the absence (○) and presence (●) of phorbol ([phorbol]/[DPPC] = 1 : 50).

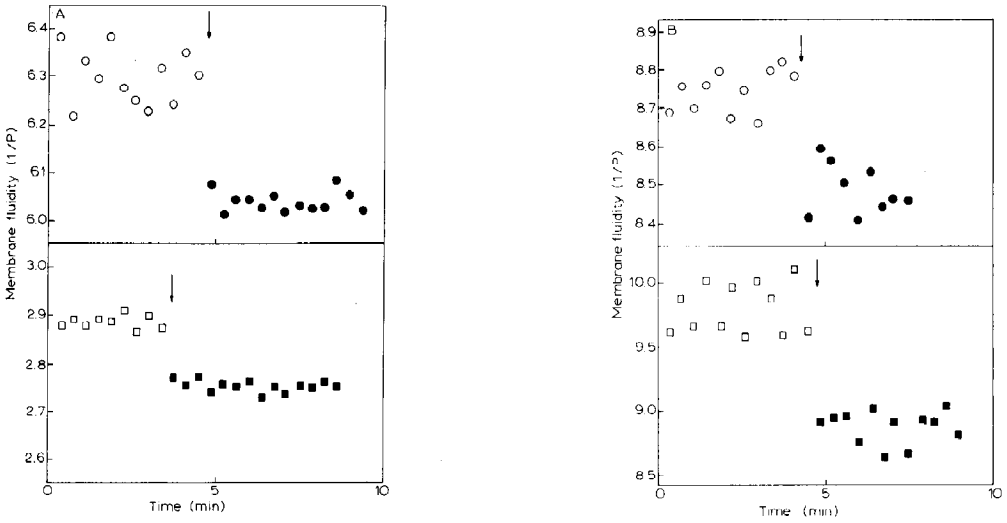

Fig. 5. Change in membrane fluidity of single unilamellar vesicles (SUV) induced by addition of teleocidin (A) below the phase transition temperature (31° C) and (B) above it (46° C). (○, ●) ANS, (□, ■) DPH. 5.2 μM teleocidin was added to 0.3 mM DPPC vesicles at the time indicated by an arrow.

perature range. TPA decreased membrane fluidity at the hydrophobic core of the lipid bilayer membrane above the phase transition temperature, whereas at the membrane surface this parameter underwent a lesser change. It is noteworthy that the trend in the results obtained is consistent with the data derived from FT-IR measurements, thus supporting the contention that TPA and teleocidin bind predominantly at the hydrophobic core and the surface of lipid bilayer membranes, respectively, and that they tend to restrict motions of lipid molecules in the membrane.

The observed changes in fluorescence depolarization are not due to quenching of fluorescent

Table 1

Change in DPPC membrane fluidity ($1/P$) induced by addition of tumor promoters

Conditions: [DPPC] = 0.3 mM, [tumor promoter] = 5.2 μM. T_c, phase transition temperature. All values are expressed as mean ± S.D. of 6 experiments. Negative values indicate a decrease in membrane fluidity.

Fluorescent probe	Tumor promoter	$T > T_c$ (45 ± 1° C)		Change in fluidity	$T < T_c$ (31 ± 1° C)		Change in fluidity
		Absence	Presence		Absence	Presence	
ANS (surface)	phorbol	9.49 ± 0.07	9.49 ± 0.04	0.00	5.92 ± 0.03	5.91 ± 0.03	−0.01
	TPA	8.85 ± 0.09	8.80 ± 0.06	−0.05	6.48 ± 0.06	6.32 ± 0.02	−0.16
	teleocidin	8.76 ± 0.08	8.60 ± 0.09	−0.16	6.28 ± 0.05	6.04 ± 0.02	−0.24
	aplysiatoxin	8.07 ± 0.04	7.98 ± 0.04	−0.09	5.88 ± 0.04	5.71 ± 0.02	−0.17
	debromo-aplysiatoxin	8.27 ± 0.07	8.00 ± 0.06	−0.27	5.77 ± 0.03	5.52 ± 0.03	−0.25
DPH (interior)	phorbol	9.78 ± 0.15	9.76 ± 0.12	−0.02	2.99 ± 0.02	2.99 ± 0.01	0.00
	TPA	9.79 ± 0.06	9.58 ± 0.12	−0.21	2.97 ± 0.007	2.96 ± 0.004	−0.01
	teleocidin	9.76 ± 0.22	9.07 ± 0.25	−0.69	2.89 ± 0.01	2.76 ± 0.01	−0.13
	aplysiatoxin	9.59 ± 0.06	9.36 ± 0.04	−0.23	2.78 ± 0.007	2.77 ± 0.003	−0.01
	debromo-aplysiatoxin	9.72 ± 0.04	9.61 ± 0.04	−0.11	2.77 ± 0.008	2.77 ± 0.006	0.00

probes by tumor promoters, since the fluorescence intensity of fluorophores underwent little change on addition of tumor promoters in the presence of DPPC SUV. For example, the change in fluorescence intensity of ANS was within the range of 3 and 1.5% for addition of TPA and teleocidin, respectively. This indicates that fluorescent probes and tumor promoters should be dispersed in membranes without forming any specific clusters, since no significant interactions occur between them. Therefore, the lifetimes of the fluorophores remained constant under the present experimental conditions, in support of the experimental data reflecting membrane fluidity.

The distribution of tumor promoters in lipid bilayer membranes was evaluated by using MLV. Tumor promoters and MLV were subjected to incubation followed by centrifugation of the suspension to obtain the supernatant. The tumor promoter content of the supernatant was assayed by HPLC on an ODS column. It was observed that about 90% of either TPA or teleocidin in a suspension bound to the lipid membrane above the phase transition temperature of the membrane and approx. 70% below this temperature. It is therefore concluded that the lipophilicity and actual concentration of tumor promoters in membranes are practically equal for both TPA and teleocidin.

Teleocidin was shown to decrease the fluidity of membranes in the gel state as judged from FT-IR and fluorescence measurements. It is known that the phosphorylcholine moiety of DPPC in membranes has an orientation parallel with the membrane surface and occupies an area of 47–54 $Å^2$, which is considerably larger than the cross-sectional area of two hydrocarbon chains of a lipid molecule in gel state membranes [37]. Therefore, if the head-group orientation is altered from being parallel to inclined upon binding of a tumor promoter to the membrane, it is possible for the hydrocarbon chains to be more closely packed as compared to the initial gel state of the membrane, resulting in the local motion of lipid molecules in the membrane experiencing restrictions due to tighter packing of the acyl chains. A suitable explanation for the effect of teleocidin below the phase transition temperature should be possible in terms of such alterations in head group orientation with respect to the matrix membrane.

Another tumor promoter, aplysiatoxin, showed behavior similar to that of TPA, indicating that it binds to a similar site on the lipid bilayer membrane as in the case of TPA. On the other hand, debromoaplysiatoxin markedly decreased the fluidity at the membrane surface, suggesting it to be located closer to the membrane surface than is TPA.

3.3. CF leakage from vesicles induced by tumor promoters

The perturbations in membrane structure induced by tumor promoter binding to lipid bilayer membranes were examined by measuring CF leakage from DPPC vesicles [22] (fig. 6).

Teleocidin, which has been shown to decrease membrane fluidity, had no effect on CF leakage from vesicles above the phase transition temperature. Below this temperature, however, an abrupt increase in CF leakage was observed immediately after the addition of teleocidin. However, steady-state leakage remained unchanged. The sharp rise in CF leakage can be explained on the basis of transient defects forming in the membrane during the initial stages of the process of tumor promoter binding to membranes. Similar observations were made in the case of debromoaplysiatoxin. In contrast, TPA and aplysiatoxin increased steady-state leakage of CF from vesicles, particularly in the case of membranes in the gel state. Notably, teleocidin and debromoaplysiatoxin, shown to bind to the membrane surface, did not enhance steady-state CF leakage from vesicles, whereas TPA and aplysiatoxin, indicated as being bound to the hydrophobic core of lipid bilayer membranes, do give rise to an increase in such leakage. Teleocidin and debromoaplysiatoxin are considered to perturb the membrane structure transiently when binding to the membrane surface, while TPA and aplysiatoxin induce formation of membrane defects after becoming embedded in the lipid bilayer membrane. The long alkyl chain present in TPA or aplysiatoxin probably facilitates deeper penetration of the hydrophobic core of a membrane by such molecules.

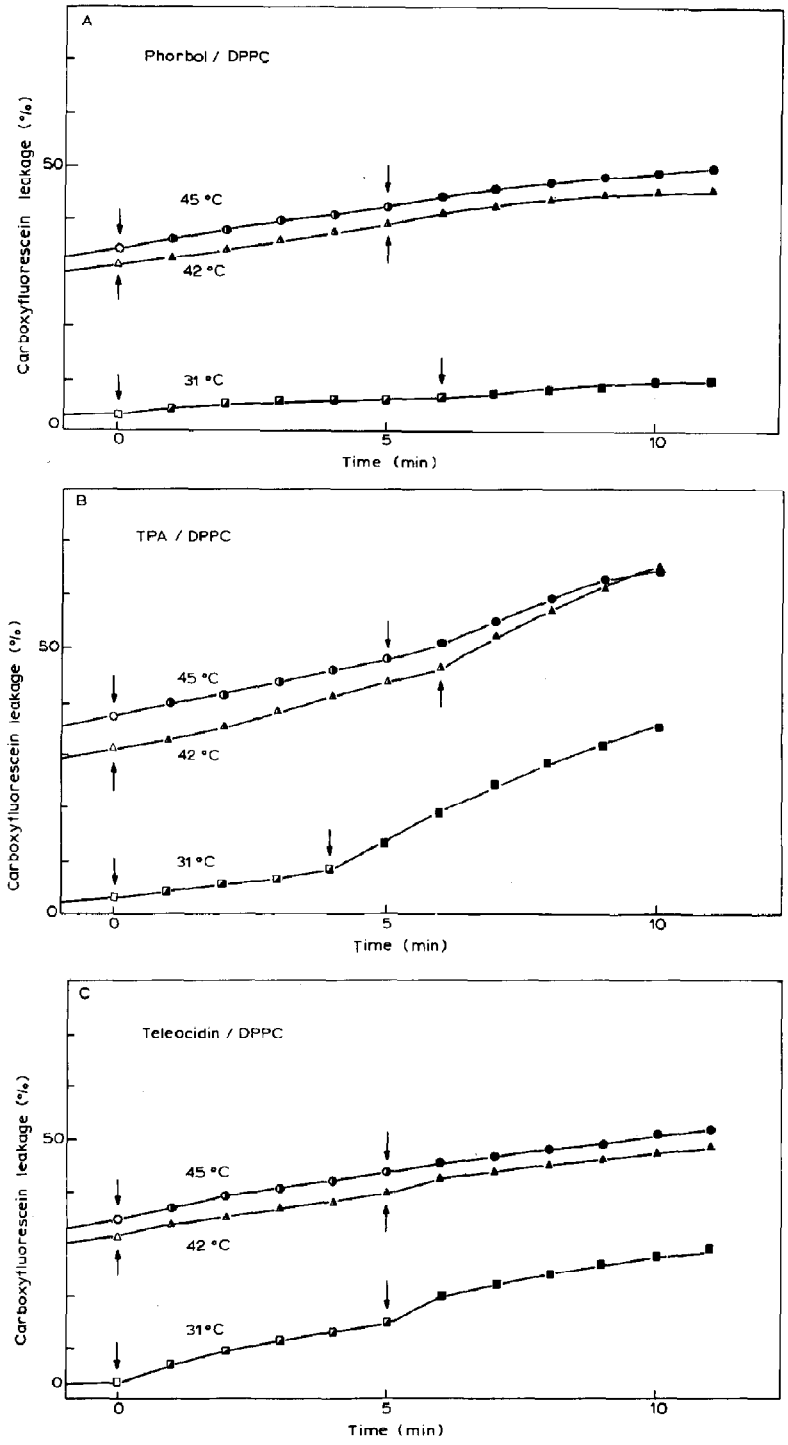

Fig. 6. CF leakage from DPPC vesicles on addition of the various tumor promoters. [DPPC] = 60 μM. Arrows indicate the first and second addition of tumor promoter at 1.0 μM (◑, ▲, ◪) and 2.1 μM (●, ▲, ■).

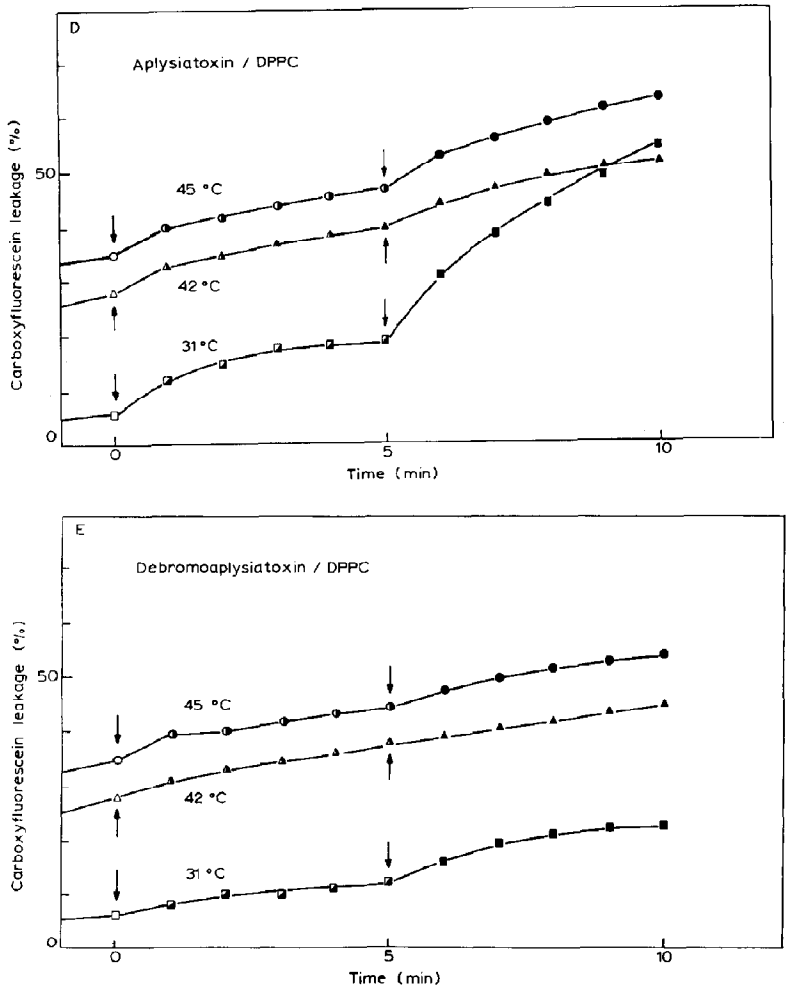

Fig. 6D and E.

The effect of tumor promoters on CF leakage from vesicles was prominent below the phase transition temperature, and therefore is not necessarily consistent with the data obtained from FT-IR and fluorescence depolarization measurements. Hence, it is possible that another effect of tumor promoters on membrane structure might be responsible for the leakage of CF. For example, it has often been pointed out that membranes in the gel state cause lateral separation of the phases into a lipid domain containing a high concentration of additive and a pure lipid domain [38]. The boundary region between the two domains is considered to act as a channel for CF leakage from vesicles. (Upreti and Jain have called such ill-defined surface defects 'cracks' [39].)

It is noteworthy that the effect on CF leakage induced by debromoaplysiatoxin, which is about 10-fold weaker than aplysiatoxin in inhibiting TPA-receptor binding, was definitely smaller as compared to aplysiatoxin. The different effect of debromoaplysiatoxin from that of aplysiatoxin on CF leakage suggests that the dipole moment of the aromatic moiety in aplysiatoxin plays a particular

role in binding to lipid bilayer membranes and that it affects the arrangement of lipid molecules in a membrane.

The significance of these results can be discussed in relation to the biological activity of tumor promoters as follows. Micromolar concentrations of tumor promoters were used in this investigation, thus being higher than those routinely employed in studies on the physiological situation. However, it is of note that dibucaine, known as a local anesthetic and supposed to exert an effect via modification of membrane fluidity [40], showed no significant effect on membrane structure at the concentrations used here. Much higher levels of dibucaine are required to induce alterations in the membrane structure as demonstrated in the present investigation. In addition, such measurements allow the detection of changes in the entire membrane structure, while being insensitive to local changes. This should therefore explain the requirement for micromolar concentrations of additives in these experiments. Accordingly, it may be stated that such tumor promoters specifically modify the membrane structure.

The present data are in support of the mechanism of activation of protein kinase C by tumor promoters proposed by Nozawa et al. [22,23]. They demonstrated that formation of a hydrophobic surface in membranes enhanced binding of protein kinase C to the membrane and induced the active conformation of the enzyme. In the present article, tumor promoters have been found to suppress the motion of lipid molecules in the membrane, supposed as having been induced by dehydration at the membrane surface. In addition, TPA is suggested to induce 'cracks' in the membrane, thereby enhancing binding of protein kinase C to the membrane and enzymatic activity [27]. Although the tumor promoters bind directly to protein kinase C and induce enzymatic activity, they should also affect the membrane structure, which is believed to be involved to some degree in the regulation of enzymatic activity.

This study reports analyses only of the interaction of tumor promoters with lipid bilayer membranes. Nevertheless, the results obtained should be of interest regarding other aspects: Firstly, the tumor promoters have been shown to exert bulk effects on membrane structure. Secondly, such effects differ between tumor promoters. The latter finding may be correlated with the differing values observed for physiological activity [6,7]. For example, the minimum concentration of tumor promoter for half-maximal activation of protein kinase C was shown to differ among tumor promoters [41], although specific binding of [^{3}H]TPA to a mouse particulate fraction was inhibited by equimolar levels of tumor promoter [2]. Furthermore, the average number of tumor cells per mouse that were induced by TPA differed from that in the case of teleocidin, and the dose-response profiles for tumor promotion by these reagents were not identical. Taken together, the differences in biological potency between these tumor promoters might be related with their differing effects on membrane structure. The enzymatic activity of some membrane proteins has been found to be influenced by TPA addition, although it does not bind to such membrane proteins. Supposedly, TPA modified the activity of the protein through changing the membrane structure. Investigation of a composite system comprising tumor promoter, membrane protein and lipid membrane is currently in progress.

References

1 I. Berenblum, Cancer Res. 1 (1941) 44.
2 H. Fujiki and T. Sugimura, Cancer Surv. 2 (1983) 539.
3 H. Fujiki, M. Suganuma, R.E. Moore and T. Sugimura, in: Human Carcinogenesis, eds. C.C. Harris and H. Autrup (Academic Press, New York, 1983) p. 305.
4 H. Fujiki, M. Suganuma, M. Nakayasu, H. Hoshino, R.E. Moore and T. Sugimura, Jap. J. Cancer Res. (Gann) 73 (1982) 495.
5 H. Nishino, K. Yoshioka, A. Iwashima, H. Takizawa, S. Konishi, H. Fujiki and T. Sugimura, Jap. J. Cancer Res. 77 (1986) 33.
6 K. Shimomura, M.G. Mullinix and K. Kakunaga, Science 222 (1983) 1242.
7 A.D. Horowitz, H. Fujiki, I.B. Weinstein, A. Jeffrey, E. Okin, R.E. Moore and T. Sugimura, Cancer Res. 43 (1983) 1529.
8 B. Weinstein, S. Gattoni-Celli, P. Kirshmeier, W. Hisao, A.D. Horowitz and A. Jeffrey, Fed. Proc. 43 (1984) 2287.
9 Y. Nishizuka, Nature 308 (1984) 693.
10 Y. Nishizuka, Science 233 (1986) 305.

11 U. Kikkawa, Y. Takai, Y. Tanaka, R. Miyake and Y. Nishizuka, J. Biol. Chem. 258 (1983) 11442.
12 S. Young, E. Chen, S. Stabel, M.D. Waterfield and A. Ullrich, Science 233 (1986) 853.
13 C.L. Ashendel, Biochim. Biophys. Acta 822 (1985) 219.
14 R.M.J. Liskamp, A.R. Brothman, J.P. Arcoleo, O.J. Miller and I.B. Weinstein, Biochem. Biophys. Res. Commun. 131 (1985) 920.
15 P.L. Tran and M.A. Deugnier, Carcinogenesis 6 (1985) 433.
16 K.B. Delclos, D.S. Nagle and P.M. Blumberg, Cell 19 (1980) 1025.
17 P.E. Driedger and P.M. Blumberg, Proc. Natl. Acad. Sci. U.S.A. 77 (1980) 567.
18 A.D. Horowitz, E. Greenebaum and I.B. Weinstein, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 2315.
19 M.D. Bazzi and G.L. Nelsestuen, Biochemistry 26 (1987) 115.
20 M.D. Bazzi and G.L. Nelsestuen, Biochemistry 26 (1987) 1974.
21 M.D. Bazzi and G.L. Nelsestuen, Biochemistry 26 (1987) 5002.
22 K. Ohki, T. Yamauchi, Y. Banno and Y. Nozawa, Biochem. Biophys. Res. Commun. 100 (1981) 321.
23 K. Ohki, T. Sekiya, T. Yamauchi and Y. Nozawa, Biochim. Biophys. Acta 693 (1982) 341.
24 C.D. Stubbs, Essays Biochem. 19 (1983) 1.
25 K.B. Delclos, E. Yeh and P.M. Blumberg, Proc. Natl. Acad. Sci. U.S.A. 80 (1983) 3054.
26 R. Schmidt, K. Heek, B. Sorg and E. Hecker, Cancer Lett. 26 (1985) 97.
27 W.J. Malaisse and M. Deleers, Colloids Surf. 10 (1984) 257.
28 E. Ralson, L.M. Hjelmeland, R.D. Klausner, J.N. Weinstein and R. Blumental, Biochim. Biophys. Acta 649 (1981) 133.
29 H. Fujiki, M. Mori, M. Nakayasu, M. Terada and T. Sugimura, Biochem. Biophys. Res. Commun. 90 (1979) 976.
30 H. Fujiki, M. Mori, M. Nakayasu, M. Terada, T. Sugimura and R.E. Moore, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 3872.
31 J. Barbet, P. Machy, A. Truneh and L.D. Leserman, Biochim. Biophys. Acta 772 (1984) 347.
32 R.K. Raheja, C. Kaur, A. Singh and I.S. Bhatia, J. Lipid Res. 14 (1973) 695.
33 D.C. Lee, A.A. Durrani and D. Chapman, Biochim. Biophys. Acta 769 (1984) 49.
34 J. Umemura, D.G. Cameron and H.H. Mantsch, Biochim. Biophys. Acta (1980) 32.
35 H.H. Mantsch, D. Madec, R.N.A.H. Lewis and R.N. McElhaney, Biochim. Biophys. Acta 980 (1989) 42.
36 R.E. Jacobs and S.H. White, Biochemistry 26 (1987) 6127.
37 H. Hauser, I. Pascher, R.H. Pearson and S. Sundell, Biochim. Biophys. Acta 650 (1981) 21.
38 A. Uemura, S. Kimura and Y. Imanishi, Biochim. Biophys. Acta 729 (1983) 28.
39 G.C. Upreti and M.K. Jain, J. Membrane Biol. 55 (1980) 113.
40 K. Saeki, Y. Nozawa, H. Shimonaka, K. Kawai, M. Ito and M. Yamamoto, Biochem. Pharmacol. 28 (1979) 1095.
41 H. Fujiki, Y. Tanaka, R. Miyake, U. Kikkawa, Y. Nishizuka and T. Sugimura, Biochem. Biophys. Res. Commun. 120 (1984) 339.