Biochimica et Biophysica Acta, 323 (1973) 647–652

BBA Report

BBA 71186

Surface behavior of dibenzanthracene with lipid monolayers

ALBERT A. BELMONTE* and JAMES SWARBRICK**

Pharmaceutics Section, School of Pharmacy, University of Connecticut, Storrs, Conn. 06268 (U.S.A.)

(Received September 21st, 1973)

SUMMARY

The surface behavior of insoluble monolayers of lecithin–cholesterol mixed films and lecithin with two structural isomers of dibenzanthracene is reported. Surface pressure and surface potential findings are discussed in view of a previous report and published results of other workers. Results showed that cholesterol may be a membrane lipid capable of a critical interaction with carcinogen leading to tumor initiation.

Polycyclic aromatic hydrocarbons are known to induce cancer in living systems[1] While this fact has been known for some time, the mechanism of cancer induction is still not known. Recent theories have suggested the possibility of cell membrane alteration as a prime factor in the initiation of the neoplastic state[2–4]. If carcinogenic hydrocarbons alter cell membrane lipids, the ability of the cell to control uptake mechanisms may be changed, thus giving rise to erratic growth behavior. It is possible this abnormal growth behavior may be an initiating step in the disease process called cancer.

Insoluble monolayers are a useful model system for mimicking biological interfaces. Although not widely used, the system offers advantages in studying interactions of molecules in fixed orientation. In a previous publication[5] we commented on the unique interaction occurring between structural isomers of dibenzanthracene and cholesterol monolayers. This report expands the previous study to include the membrane lipid L-α-β, γ-dipalmitoyl phosphatidylcholine (lecithin), and mixed monolayers of lecithin–

* Present address: Auburn University, School of Pharmacy, Auburn, Ala. 36830, U.S.A. To whom all correspondence should be addressed.
** Present address: Sterling–Winthrop Research Institute, Rensselaer, N.Y. 12144, U.S.A.

cholesterol. This was done in hopes of elucidating additional aspects of the surface chemistry of dibenzanthracene and its behavior at the air–water interface.

Previous studies have shown that polycyclic aromatic hydrocarbons interact with membrane lipids[6–8]. While results of studies seem somewhat consistent, biological interpretation remains unclear. Dibenzanthracene was chosen for study for several reasons. First, the hydrocarbon has two structural isomers which are very different in their reactions *in vivo*. One isomer, 1,2,3,4-dibenzanthracene, is non-carcinogenic or weakly carcinogenic; while another isomer, 1,2,5,6-dibenzanthracene is strongly carcinogenic[9]. Second, dibenzanthracene has been studied extensively and its *in vivo* behavior well characterized. By utilizing these facts it was hoped that more meaningful biological interpretation could be attached to this *in vitro* system.

Snart[10] and Weiner *et al.*[11] have shown significant interactions of membrane lipids with some polycyclic aromatic hydrocarbons using the monolayer technique. More recently Felmeister *et al.*[12] have reported on the interaction of 3,4-benzpyrene with cholesterol, lecithin, proteins and lipid–protein films. While these studies support hydrocarbon–lipid interaction, extrapolation to the *in vivo* situation appears to be lacking. Additionally, no use was made of the fact that some of the carcinogenic hydrocarbons studied have non-carcinogenic isomers. The present study, contrasting a carcinogenic and non-carcinogenic isomer with membrane lipids, was undertaken in an attempt to provide insight into *in vivo* interactions with these compounds.

The compounds used in the study were cholesterol (S.C.W., Nutritional Biochemical Corp., Cleveland, Ohio), L-α-β,γ-dipalmitoyl phosphatidylcholine (lecithin) (Calbiochem Los Angeles, Calif.), 1,2,3,4-dibenzanthracene (N.B.C.), and 1,2,5,6-dibenzanthracene (N.B.C.). All compounds were submitted to thin-layer chromatography using silica gel G and H plates in polar and non-polar solvents and found to be chromatographically pure.

Mixed films were prepared by dissolving the lipids in chloroform (Nanograde–Mallinckrodt, St. Louis, Mo.) and the hydrocarbons in benzene (Nanograde). The appropriate molar ratios of hydrocarbon:lipid and hydrocarbon:mixed lipid were prepared using a calibrated syringe (Digi-Pet, Manostat Corp., New York, N.Y.). The pre-mixed solution was used as the film-forming material. The solutions were spread on the film balance in a drop-wise fashion using a micropipet (Bio-Rad Laboratories, New York, N.Y.). When not in use the film-forming solutions were stored at 18 °C. Double distilled water from an all-glass still was used as the subphase. Normal saline was prepared by adding analytical reagent grade NaCl to the double distilled water.

Details of the Wilhelmy-type film balance, for measuring surface pressure, and the apparatus for determining surface potential have been described previously[13,14].

The film balance trough was filled with subphase (water or saline) at 25 °C and allowed to equilibrate for 15 min; the surface was then swept clean. The film-forming solution was pipetted onto the aqueous surface and allowed to spread. After 10 min, compression was carried out at a rate of 2.54 $cm \cdot min^{-1}$. Data was collected simultaneously from surface pressure and surface potential recordings to product Π–A and ΔV–A isotherm respectively. All runs were performed in triplicate at 25 °C.

Neither 1,2,3,4-dibenzanthracene nor 1,2,5,6-dibenzanthracene form films when spread from benzene or other commonly used spreading solvents. No surface pressure was generated when various concentrations of the hydrocarbons in benzene were deposited on aqueous subphases; nor when solutions of hydrocarbon were injected into the subphase. This is not unexpected, since these hydrocarbons do not possess any hydrophilic groups. Accordingly, all studies were carried out with mixtures of the hydrocarbon and lipids as described earlier.

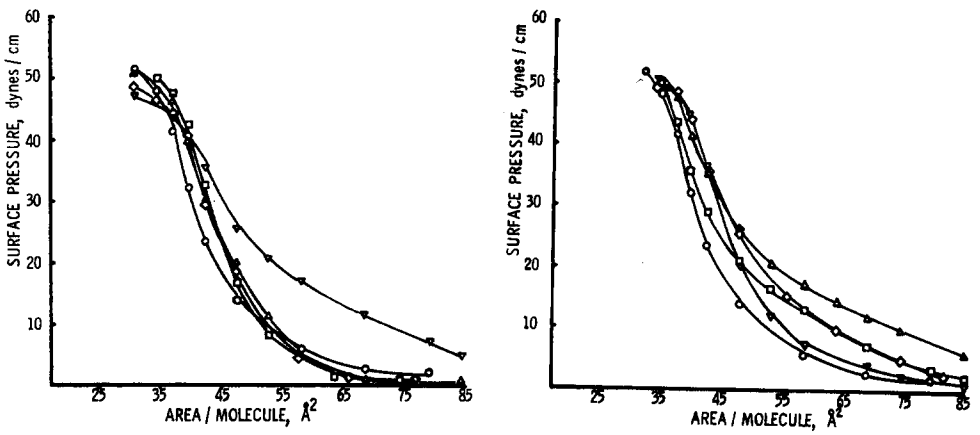

Fig. 1. Interaction of 1,2,3,4-dibenzanthracene with lecithin/cholesterol on saline. ○, lecithin–cholesterol (3:1); □, dibenzanthracene: lecithin–cholesterol (1:10); △, dibenzanthracene: lecithin–cholesterol (1:1); ◇, dibenzanthracene: lecithin–cholesterol (3:1); ▽, dibenzanthracene: lecithin–cholesterol (5:1).

Fig. 2. Interaction of 1,2,5,6-dibenzanthracene with lecithin/cholesterol on saline. ○, Lecithin–cholesterol (3:1); □, dibenzanthracene: lecithin–cholesterol (1:10); △, dibenzanthracene: lecithin–cholesterol (1:1); ◇, dibenzanthracene: lecithin–cholesterol (3:1); ▽, dibenzanthracene: lecithin–cholesterol (5:1).

Fig. 1 shows the interaction of 1,2,3,4-dibenzanthracene with lecithin–cholesterol monolayers on saline. The lipids (lecithin–cholesterol) were present as a 3:1 molar mixture, respectively. There is some interaction between the hydrocarbon and the mixed lipid film as evidenced by the more expanded isotherms, the interaction being greatest at the highest ratio of hydrocarbon to lecithin–cholesterol (5:1). The upper break of the isotherms, which occurs at approximately 40 Å ^{2} (based on total lipid present) indicates a mutual solubility of lipids and hydrocarbon. Since this break is also present with the lecithin–cholesterol isotherm (where no hydrocarbon is added), it is probably due to mutual solution of the lipids. The 5:1 hydrocarbon:lipid film shows a marked interaction. The other isotherms produced from less concentrated hydrocarbon:lipid films indicate that, over the range from a 1:10 to 3:1 molar ratio of hydrocarbon:lipid, the extent of interaction is the same.

The interactions of lecithin–cholesterol monolayers with the potent carcinogen 1,2,5,6-dibenzanthracene are shown in Fig. 2. Several differences are discernible when compared with Fig. 1. Thus, in Fig. 2, the extent of interaction at high surface pressures lies

between that for the control isotherm (lecithin–cholesterol) and those for the other concentrations. This may be contrasted to the isotherms produced from 1,2,3,4-dibenzanthracene and lipid shown in Fig. 1 where the extent of interaction was the same. Additionally at lower surface pressures, the film showing the greatest interaction is the 1:1 ratio film.

Differences and varying trends in behavior of the hydrocarbon: mixed lipid films make interpretation difficult, even though surface potential measurements (ΔV) were also obtained. Since a unique surface behavior had been seen previously when the dibenzanthracene interacted with cholesterol[5], the behavior of these hydrocarbons with lecithin was not assumed to be merely a differential between lecithin–cholesterol and cholesterol monolayer behavior. Therefore, the interaction of these hydrocarbons with lecithin alone was also investigated.

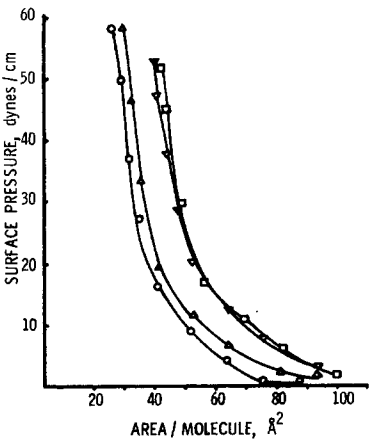

Fig.3. Surface pressure–area per molecule relationship for lecithin and dibenzanthracene: lecithin films. ○, lecithin on saline; △, lecithin on water; □, 1,2,3,4-dibenzanthracene: lecithin on water; ▽, 1,2,5,6-dibenzanthracene: lecithin on water;

Fig. 3 shows the compression isotherms for lecithin on water and saline. When compared to water, there is a condensing effect on saline. Thus, the cross-sectional area per molecule for lecithin (extrapolated to $\Pi = 0$) on water was found to be 44 Å^2 (ΔV = 560 mV), while on 0.9% NaCl a cross-sectional area per molecule of 39 Å^2 (ΔV = 535 mV) was found. Fig. 3 also shows the results for 1,2,3,4-dibenzanthracene–lecithin and 1,2,5,6-dibenzanthracene–lecithin interactions when monolayers were compressed on water. There was no difference between various concentrations of hydrocarbon:lecithin employed. Indeed there appears to be only a slight difference, if any, between the interaction of the two compounds with lecithin.

Surface potential data showed large variations in these systems, due probably to excess dibenzanthracene and film inhomogeneity. This is supported by the surface pressure isotherms which show lecithin bound both isomers of dibenzanthracene to about the same extent and at all concentrations employed. Maximum binding was found with the 1:10 dibenzanthracene:lecithin film. At concentrations greater than 1:10, the additional

hydrocarbon does not bind and may remain at the interface as excess material.

Figs 1 and 2 show the effect of the addition of the dibenzanthracenes to lecithin–cholesterol films prior to spreading. With the exception of the 5:1 film, very little surface interaction occurs between 1,2,3,4-dibenzanthracene and the mixed film. However, even with the 5:1 film, interaction is weak and excess material is extruded from the surface as compression is carried out.

A different interaction dependent on the concentration of the hydrocarbon is observed between 1,2,5,6-dibenzanthracene and lecithin–cholesterol films. Fig. 2 shows the 1:1 film of 1,2,5,6-dibenzanthracene:lecithin–cholesterol interacts to the greatest extent, as is evident by the greatest expansion with this film when compared to the others. Although the interaction appears weak, there is incomplete expulsion of the hydrocarbon from the monolayer, even at high surface pressures. The isotherms do not revert entirely to the control lecithin–cholesterol isotherm, indicating alteration of the lipids and association between hydrocarbon and lipid.

Fig. 3 supports the concept that lecithin has the ability to bind amounts of hydrocarbon strongly. This is important as *in vivo* considerations indicate that cell membranes have binding capacity. The absence of extrusion or squeezing out of hydrocarbon from the monolayer may thus have profound importance. If extrusion can be compared to desorption from the monolayer, then the ability of lecithin to bind and keep hydrocarbon at the interface may inhibit further penetration of the hydrocarbon into the cell.

The ability for a hydrocarbon to desorb from the interface is important, as this may be considered as a method of penetration. The first step of the interaction to consider is whether a compound can interact with lipid and cause association at the interface. Second, whether the material dissociates readily, leaving the lipid essentially intact, *i.e.* no chemical reaction. This overall process can be viewed as a two-step physical penetration model. The desorption or dissociation occurring at low areas or high surface pressures is important biologically since living cell membranes are considered to exhibit a surface pressure of approximately 30 dynes$\cdot$cm^{-1}. This behavior was readily observed with cholesterol monolayers and 1,2,5,6-dibenzanthracene as previously reported[5], while 1,2,3,4-dibenzanthracene (the non-carcinogen) did not show association with cholesterol. With lecithin monolayers both dibenzanthracenes showed a strong association, desorption not occurring even at high surface pressures. The lecithin–cholesterol mixed film studies showed hydrocarbon behavior intermediate to that described for each of the pure lipid films.

The strong binding of lecithin may be a protective mechanism of cell membranes, thereby preventing entrance of material into the cell. This is consistent with the work of Altman[15] who has reported that lecithin has a protective effect against cell tumors induced by polycyclic aromatic hydrocarbons. If this is so, then cholesterol interactions take on major importance in tumor initiation by polycyclic aromatic hydrocarbons, and penetration of the cell membrane by these materials.

This work was supported by Grant 117 from the University of Connecticut Research Foundation, Storrs, Conn. 06268, U.S.A.

REFERENCES

1 Cook, J.W., Hieger, I., Kennaway, E.L. and Mayneord, W.V. (1932) *Proc. R. Soc.* B111, 455–484
2 Weissmann, G., Troll, W., Van Duuren, B.L. and Sessa, G. (1968) *Biochem. Pharmacol.* 17, 2421–2434
3 Kubinski, H., Andersen, P.R. and Kellicut, L.M. (1972) *Chem. Biol. Interact.* 5, 279–283
4 Holley, R.W. (1972) *Proc. Natl. Acad. Sci. U.S.* 69, 2840–2841
5 Belmonte, A.A. and Swarbrick, J. (1973) *J. Pharm. Sci.* 62, 481–483
6 Clowes, G.H.A., Davis, W.W. and Krahl, M.E. (1939) *Am. J. Cancer* 37, 453–454
7 Davis, W.W., Krahl, M.E. and Clowes, G.H.A. (1940) *J. Am. Chem. Soc.* 62, 3080–3098
8 Davis, W.W., Krahl, M.E. and Clowes, G.H.A. (1942) *J. Am. Chem. Soc.* 64, 108–110
9 Heidelberger, C. (1970) *Eur. J. Cancer* 6, 161–172
10 Snart, R.S. (1967) *Biochim. Biophys. Acta* 144, 10–17
11 Weiner, N.D., Chawdry, I. and Felmeister, A. (1971) *J. Pharm. Sci.* 60, 425–428
12 Felmeister, A., Tsai, D. and Weiner, N.D. (1972) *J. Pharm. Sci.* 61, 1065–1068
13 Munden, J.W., Blois, D.W. and Swarbrick, J. (1969) *J. Pharm. Sci.* 58, 1308–1312
14 Blois, D.W. and Swarbrick, J. (1972) *J. Pharm. Sci.* 61, 393–399
15 Altman, R.F.A. (1968) *Arch. Geschwulstforsch.* 31/2, S133–143