Biomimetic Approach to Biomembrane Models Studies: Medium Influence on the Interaction Kinetics of Some Phenylurea Derivatives Herbicides

ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · MAY 2006

Impact Factor: 5.33 · DOI: 10.1021/es0518373 · Source: PubMed

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Environ. Sci. Technol. **2002**, *36,* 2717−2723

Calorimetric Approach of the Interaction and Absorption of Polycyclic Aromatic Hydrocarbons with Model Membranes

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The ability of polycyclic aromatic hydrocarbons (PAHs) to interact with cell membranes outer lipid layer and subsequently to penetrate inside cells can be a prerequisite for exhibiting a mutagenic and carcinogenic activity. The effect exerted by pyrene, benzo[*a*]pyrene, and anthracene, three structurally similar polycyclic aromatic hydrocarbons possessing mutagenic and carcinogenic activity on the thermotropic behavior of model membranes represented by dimyristoylphosphatidylcholine (DMPC) vesicles, was investigated by differential scanning calorimetry (DSC).The examined compounds, when dispersed in liposomes during their preparation, exerted a different action on the gel-to-liquid crystal phase transition of DMPC multilamellar vesicles. Pyrene and benzo[*a*]pyrene affected the transition temperature (T_m), shifting it toward lower values with a concomitant decrease of the associated enthalpy changes (ΔH). Anthracene does not significantly affect the thermotropic behavior of lipid vesicles for all tested concentrations. The interaction between PAHs and model membranes was also studied by considering the ability of such compounds as a finely powdered solid or adsorbed on soil surrogate (constituted by silica gel) to migrate through an aqueous medium. This transfer process was compared with the PAHs intermembrane transfer from PAH loaded liposomes to empty membranes. These processes can mimic absorption kinetics mediated by hydrophilic or lipophilic media. No interaction occurred between model membranes and solid PAHs. A very small effect was also observed for PAHs released by silica gel, suggesting that the migration and absorption are hindered by the aqueous layer and that their low hydrophilic character inhibits migration through the aqueous layer surrounding the multilamellar vesicles (MLV). Different behavior was observed by considering the time-dependent studies carried out by contacting, for increasing times, equivalent amounts of empty DMPC vesicles with PAH loaded ones; all compounds were able to migrate between the two different kinds of model membranes. Thus, PAHs are unable to reach and penetrate biological membranes migrating through an aqueous layer but, when dispersed in a lipophilic medium, are able to penetrate and diffuse inside a membrane. The obtained experimental results seem to validate the employment of the DSC technique in order to study the ability of bioactive compounds, not only to interact with biological membranes, but also to be adsorbed inside a cell when dispersed in a lipophilic medium.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are molecules possessing a condensed ring skeleton, and some of them are mutagenic and carcinogenic substances formed during the incomplete burning of coal, oil and gas, garbage, or other organic substances such as tobacco or charbroiled meat (*1*−*3*).

PAHs, emitted from both natural and anthropogenic processes, are persistent organic pollutants due to their chemical stability and biodegradation resistance. While inhaled, PAHs have long been suspected to induce lung cancer in humans (*4*). They are able to pass through the skin, even though the percoutaneous penetration is affected by various factors connected to exposure conditions (*5*).

PAHs containing a bay or bay-like region were shown to be potent inhibitors of gap−junction intercellular communication, an epigenetic event involved in the removal of an initiated cell from growth suppression (*6*). Pyrene may promote allergic diseases by inducing the production of IL-4 (*7*). Benzo[*a*]pyrene causes cancer in laboratory animals when applied to their skin (*8*). Benzo[*a*]pyrene produces immunotoxicity by increasing the intracellular Ca^{2+} in lymphocytes (*9*). It has anticholinesterase activity (*10*) and can influence the development of different steroid receptors (*11*). It is a potent small intestinal mutagen in mice (*12*). Anthracene is acutely toxic to plants and fish exposed to solar ultraviolet radiation of environmentally realistic intensities. Its toxicity appears associated with a reactive oxygen radical-induced lipid peroxidation and with a general disruption of cell membrane function (*13, 14*), it also inhibits the photosynthesis in natural assemblages of phytoplankton exposed to solar radiation (*15*), and it has an effect on the allergen-specific IgE and IgG1 response in mice (*16*).

While the organization and interaction of PAHs in membrane systems are studied, it is important to assess their capacity to be absorbed by a cell membrane, and it is well-known that such compounds, to exert their potential carcinogenic effect, should be introduced inside cells.

Differential scanning calorimetry (DSC) is a technique employed to carry out studies on the effect of increasing incorporation of bioactive compounds in lipid bilayers. This technique permits the study of the effect exerted by a bioactive substance, when dispersed in a biological membrane, on the gel-to-liquid crystal phase transition ($L_\beta - L_\alpha$), exhibited by phospholipid species when heated (*17*−*21*). Usually, a substance dissolved in a lipid layer decreases its transitional temperature (*22, 23*). The magnitude of the destabilizing effect is related to the amount of chemicals dissolved in the lipid structure. By DSC, it is also possible to monitor the uptake process of a compound in a model membrane surface. The compound lipophilicity and water solubility, as well as the phospholipid membrane composition and phase separation presence (*24*−*26*), modulate this process.

In this work, the interaction of PAHs with L-α-dimyristoylphosphatidylcholine (DMPC) vesicles are investigated, as previously described by studying the interaction of structurally similar compounds (*25*−*27*) with DMPC multilamellar vesicles. These vesicles are usually employed as

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10.1021/es010260w CCC: $22.00
Published on Web 05/02/2002

synthetic simplified model membranes showing a change in their thermotropic behavior when foreign molecules are dissolved in their ordered structure (*23*, *28*, *29*). Such a "classical" method of investigating the interaction between bioactive compounds and membranes (interaction in organic solvent before multilamellar vesicles preparation) can be considered as only a way to report the maximum interaction between a foreign molecule and lipidic bilayer. This interaction is mainly affected by the partition of the compounds between the lipid phase and the aqueous phase. However, following such a procedure it is not possible to obtain information on the real ability of a compound to permeate the aqueous layer surrounding the lipid membrane or to pass through the lipid bilayer to penetrate into a cell-like model. A way to demonstrate such processes (*24*, *26*, *27*, *30*−*32*) is the comparison of the data obtained through the aforementioned "classical" method with kinetic experiments of the transfer of compounds to empty membranes through aqueous medium and of the transfer of PAHs, dispersed in lipid multilayers, to empty membranes. In this way, it is possible to detect differences in the compound's ability to interact and penetrate the lipid bilayer of biomembranes, which causes variations in their packing and fluidity.

Experimental Section

Materials. Pyrene (purity, 97.4%) was purchased from Supelco (Bellefonte, PA). Benzo[*a*]pyrene (purity, 97%) and anthracene (purity, 99%) were obtained from Aldrich (Germany). Synthetic L-α-dimyristoylphosphatidylcholine was obtained from Fluka Chemical Co. (Buchs, Switzerland).

Silica gel type W 254 with a surface of 540 m^2/g was obtained from Grace (Gmbh) (Germany) and treated at 350 °C in nitrogen flow before use.

Lipid was chromatographically pure as assessed by two-dimensional thin-layer chromatography (TLC). Lipid concentrations were determined by phosphorus analysis (*33*). Buffer solution consisted of 50 mM Tris, adjusted to pH = 7.4 with hydrochloric acid.

Liposomes Preparation. Multilamellar vesicles (MLV) were prepared in the presence and absence of anthracene, pyrene, or benzo[*a*]pyrene. Chloroform stock solutions of lipid and PAH were mixed to obtain increasing molar fraction of compounds. The solvent was removed under nitrogen flow, and the resulting film was freeze-dried to remove the residual solvent. Liposomes were prepared by adding to the films 50 mM Tris buffer solution (pH = 7.4), heating at 37 °C (temperature above the gel−liquid crystalline phase transition), and vortexing 3 times for 1 min.

The samples were shaken for 1 h in a water bath at 37 °C to homogenize the liposomes permitting the compounds to redistribute between lipid and aqueous phases. Then, aliquots of 120 μL of lipid suspensions (5 mg of lipid) were transferred in a 160 μL DSC aluminum pan, hermetically sealed, and submitted to DSC analysis.

DSC. DSC was performed by using a Mettler TC-15 system equipped with a DSC-30 calorimetric cell and a Mettler STAR V 6.10 SW software. The scan heating rate employed was 2 °C/min in the temperature range 5−37 °C. The sensitivity was automatically chosen as the maximum possible (around 1 mW), and the reference pan was filled with Tris buffer solution. The calorimetric system was calibrated by using indium, stearic acid, and cyclohexane. Indium and palmitic acid were also employed to calibrate the enthalpy changes (ΔH), evaluated from the peak areas using the integration program of the TA STAR Software. The calculated areas lie within the experimental error (±5%).

The samples, for the study of the interaction of increasing PAH molar fractions with liposomes, were cooled and heated 4 times to check the reproducibility of results. After calorimetric scans, aliquots of all samples were extracted from calorimetric aluminum pans to determine, by the phosphorus assay (*33*), the exact amount of phospholipid present in each sample.

Soil Surrogate Preparation. A weighed amount of each PAH was dissolved in chloroform and added to Silica gel W-254, vigorously mixed, and freeze-dried under high vacuum to eliminate the organic solvent. Aliquots of silica gel carrying adsorbed PAH were considered to determine the concentration of PAH (mmol/mg). The amount of PAH present on the silica gel was detected by UV spectroscopy at 240 nm for pyrene, 263 nm for benzo[*a*]pyrene, 253 nm for anthracene.

Permeation Experiments. The ability of examined PAHs to dissolve in the aqueous phase, migrate through it to be absorbed on the external lipidic layer of multilamellar vesicles, and successively to be transferred in the deeper layers was investigated by carrying out the following "kinetic" experiment. Fixed amounts (to obtain a 0.06 molar fraction of each PAH with respect to the phospholipid) of finely powdered PAH or supported on Silica gel was placed in the bottom of the 160 μL DSC aluminum crucible, and then a fixed amount (5 mg) of DMPC aqueous dispersion (MLV) was added. The samples, hermetically sealed in the pans, were gently shaken for 10 s and then submitted to subsequent calorimetric cycles by using the following procedure: (1) a scan between 5 and 37 °C, to detect the interaction between the compounds and the model membrane during the sample heating; (2) an isothermal period (1 h) at 37 °C to allow the PAH to dissolve in the aqueous medium (or to be released by the substrate) and possibly interact and permeate the lipid layers, which at 37 °C exist in a disordered state as they are over the lipid transitional temperature; and (3) a cooling scan between 37 and 5 °C, at the rate of 4 °C/min, to bring back the lipid layers in an ordered state before restarting the heating program (step 1).

This procedure was run at least 6 times in order to follow eventual variations of the transitional temperature of the DMPC calorimetric peak due to the release of the PAH by the solid or by silica gel to the model membrane. These calorimetric scans were repeated also for a long incubation time (12 h), to observe possible further variations in the peak temperature of the membrane target. If an interaction or a full lipid layer penetration did occur, then an effect similar to that detected when the preparation (0.06 molar fraction) carried out in organic solvent was examined should be observed.

Transmembrane Transfer Kinetics. Sometimes, lipophilic media instead of aqueous medium can support the absorption of nonpolar bioactive compounds by biological membranes. Therefore, it is interesting to investigate how the examined PAHs dispersed in MLV (previously loaded at a 0.12 molar fractions with respect to the aqueous lipid dispersion) can be transferred to empty multilamellar vesicles. If a compound transfer occurs, then an effect similar to that observed in the organic preparation at a 0.06 molar fraction of PAH will be induced. This effect is due to the emptying of the loaded membrane and the contemporary PAHs absorption inside the empty MLVs. These kinetic experiments were carried out by transferring 60 μL of empty DMPC aqueous suspension (MLV) in a 160 μL calorimetric pan where 60 μL of MLV loaded with a 0.12 molar fraction of PAH had previously been placed. The samples were hermetically sealed in the pans, gently shaken for 10 s, and then submitted to subsequent calorimetric cycles by using the same step procedure reported in the previous section. In this case, during the first step (the scan between 5 and 37 °C), the interaction between loaded liposomes and empty model membranes during the heating of the sample was detected due to the compounds transfer from loaded liposomes to membrane surface of empty liposomes.

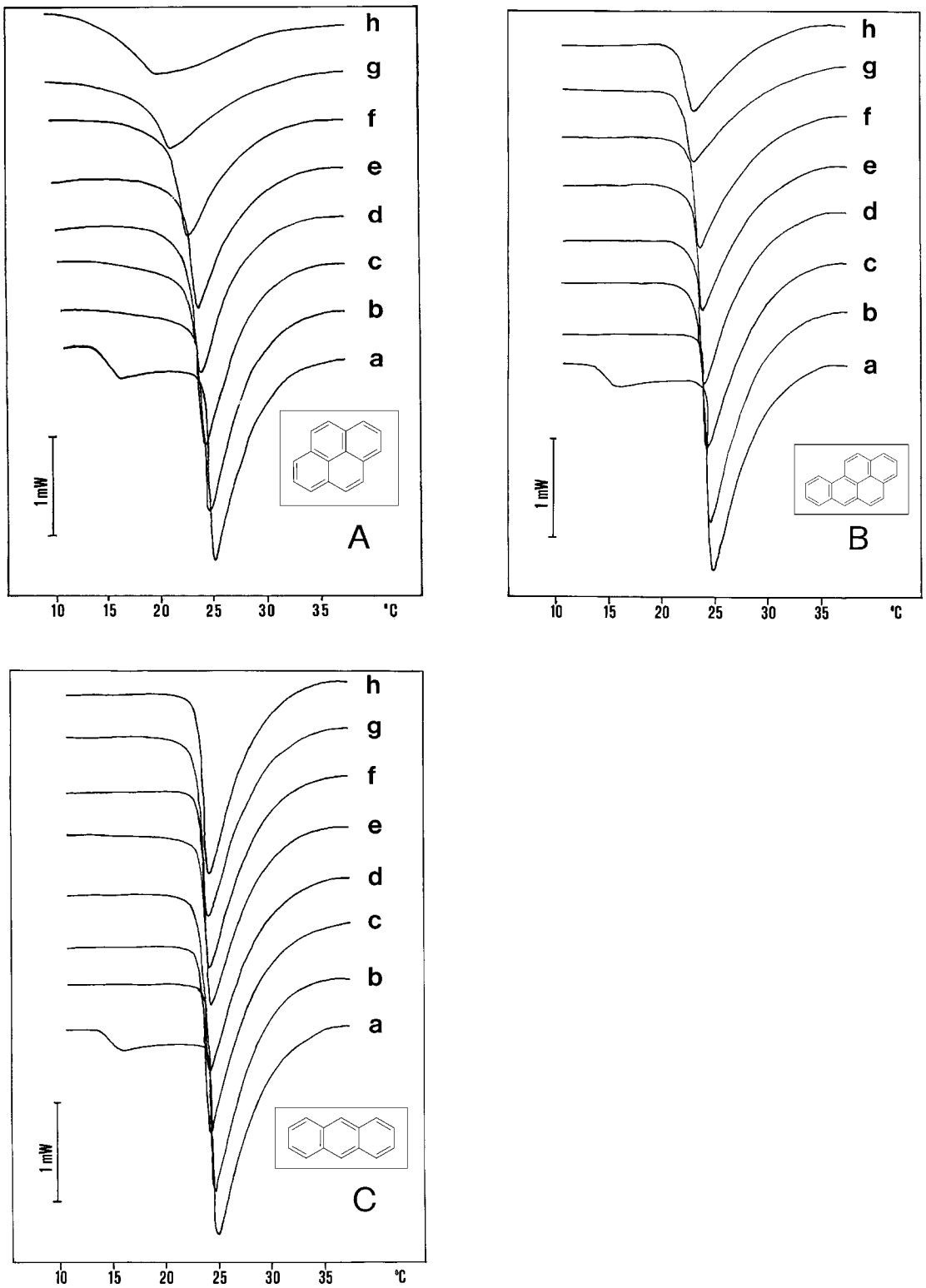

FIGURE 1. Differential scanning calorimetry heating curves of hydrated DMPC containing (A) pyrene, (B) benzo[*a*]pyrene, and (C) anthracene, obtained starting from organic solvent solutions, at a PAH molar fraction of a = 0.0, b = 0.015, c = 0.030, d = 0.045, e = 0.06, f = 0.09, g = 0.12, and h = 0.15.

Results and Discussion

The interaction between bioactive molecules and MLV vesicles can be explained in terms of a "fluidifying" effect due to the perturbation effect caused by the introduction of lipophilic molecules into the ordered structure of the lipid bilayer. Depending on the localization of the perturbing molecule, several effects can be observed. The molecules acting as spacers in lipidic structures cause a destabilization of the lipid mosaic with a decrease in the T_m of the gel-to-liquid crystal phase transition. The concomitant variation in ΔH for pyrene and benzo[*a*]pyrene can be explained as a molecular interaction with lipids in liposomes acting as "substitutional impurities" of a membrane, taking the place of lipid molecules, and such an effect can cause T_m variation

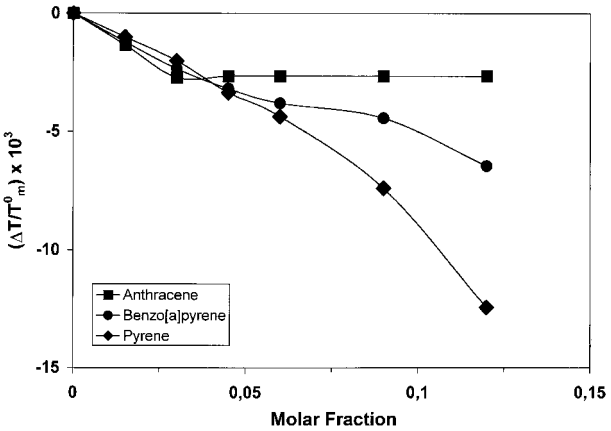

FIGURE 2. Transitional temperature variation (as $\Delta T/T_m°$) values, in heating mode, as a function of pyrene, benzo[*a*]pyrene, and anthracene molar fractions.

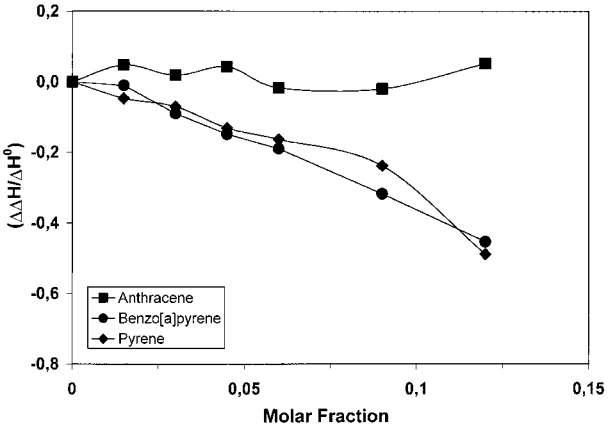

FIGURE 3. Transitional enthalpy variation (as $\Delta\Delta H/\Delta H°$) values, in heating mode, as a function of pyrene, benzo[*a*]pyrene, and anthracene molar fractions.

and ΔH change. Instead, molecules which act as "interstitial impurities", by intercalating among the flexible acyl chain of lipids, cause T_m variations without ΔH change (anthracene), according to the temperature depression of melting point for an ideal solution (*28*, *34*−*37*).

The aqueous dispersions of pure DMPC multilamellar vesicles as well as those obtained in the presence of increasing molar fractions of structurally similar PAHs (anthracene, pyrene, and benzo[*a*]pyrene), submitted to DSC scans, give different calorimetric curves (see Figure 1, parts A−C). The experimental values show that their interaction with model membranes was favored by complete dissolution in organic solvents of increasing molar fractions in the presence of a fixed amount of DMPC. These interactions were defined as the interaction at infinite time (t_{inf}). The calorimetric heating curves of DMPC−MLV in the presence of increasing pyrene, benzo[*a*]pyrene, and anthracene molar fractions show that the examined compounds are able to interact with ordered lipid bilayers, causing the disappearance of the typical pretransitional peak exhibited by DMPC liposomes as well as the shift of the calorimetric peak toward lower temperature values. The transitional temperature (T_m) shift is comparable among the PAHs up to a molar fraction of 0.03; above this value, the T_m shift was differentiated. In fact, while pyrene shows the greatest T_m decrease for all tested molar fractions, benzo[*a*]pyrene exerts such a fluidifying effect in a less evident way, whereas anthracene slightly affected the order of the model membrane. These results are better shown in Figure 2, where the temperature shifts are reported as $(\Delta T \times 10^3)/T_m°$ ($\Delta T = T_m - T_m°$, where T_m is the value of transitional temperatures obtained for each PAH/DMPC aqueous dispersion, while $T_m°$ is the transitional temperature of pure DMPC), in function of PAH molar fractions present in the aqueous lipid dispersion.

The values were obtained from experiments carried out in triplicate, and for each value of T_m, the standard deviation was less than 1.5%; thus, no statistical treatment of the data was reported.

The previous considerations on the localization of the interaction exerted by the examined PAHs are supported also by the different shape of the calorimetric curves of the studied compounds (see Figure 1A−C). In fact, by increasing the molar fraction, pyrene is able to modify the shape of the calorimetric curves with the result that, at a higher molar fraction, the curves are broadened (there is an increase in the difference of the temperatures between the starting and ending of the transitional process). A similar effect is evident for benzo[*a*]pyrene (see Figure 1B), even if in a less evident way with respect to pyrene; and it is not at all observed for anthracene, where (see Figure 1C) the shape of the curves remains nearly unmodified with respect to pure DMPC. These results are also reflected in Figure 3, where the enthalpy changes (ΔH), calculated from the calorimetric peak area, are reported as $(\Delta\Delta H)/\Delta H°$ ($\Delta\Delta H = \Delta H - \Delta H°$, where ΔH are the enthalpy changes obtained for increasing PAHs molar fractions and $\Delta H°$ is the enthalpy change of the peak associated to pure DMPC), as a function of the molar fraction of PAH present in the lipid aqueous dispersion.

DSC technique can be employed to investigate not only the potential of molecules to interact with a model membrane's bilayer but also their solubilization in an aqueous medium and the successive permeation of lipid liposomes, passing through lipid bilayers. So, it is possible to discern the eventual ability of species to migrate through the aqueous layer from the outer lipid layer to the inner lipid layer in a multilayered vesicle (*25*, *26*, *38*). PAHs, as reported previously, are able to react with the membrane model when uniformly dispersed in organic solvent during liposomes preparation, but evidence of their ability to be transferred or absorbed can be obtained only by considering the kinetic experiments. In these experiments, solid PAHs or PAHs adsorbed in silica gel or PAHs loaded in liposomes were left in contact with empty vesicles, at increasing incubation periods.

In Figure 4, parts A−C, are reported the calorimetric curves of the experiments carried out by mixing equimolar amounts of loaded liposomes (containing a 0.12 molar fraction of PAHs) with empty liposomes, for increasing incubation times. The effects of the compounds released by loaded liposomes to the empty vesicles are compared with those curves of pure DMPC as well as of DMPC loaded with 0.06 PAHs molar fraction. It is interesting that the first calorimetric curve (curves b in Figure 4, parts A and B), obtained after mixing loaded and unloaded liposomes, shows a double peak. The first one (falling at higher temperature) can be attributed to the pure DMPC; the second one (at lower temperature) is similar to that of a 0.12 molar fraction loaded vesicles. After a 1-h incubation, at 37 °C, the successive calorimetric curve shows a single peak (curves c in Figure 4, parts A and B) located at an intermediate temperature between the two peaks described previously. Such curves are shifted with time to lower temperatures, eventually overlapping to the reference curves (that obtained when DMPC MLV in the presence of a 0.06 molar fraction of pyrene or benzo[*a*]pyrene were submitted to a calorimetric scan). Anthracene (Figure 4C) exerted an effect similar to that of the other two compounds without causing a net splitting of the calorimetric curves (curve c in Figure 4C) but reaching, similarly the other PAHs, the value of 0.06 molar fraction. In Figure 5, the T_m shift of the kinetic experiments are reported and compared with the data obtained for the MLV prepared in the presence of a 0.06

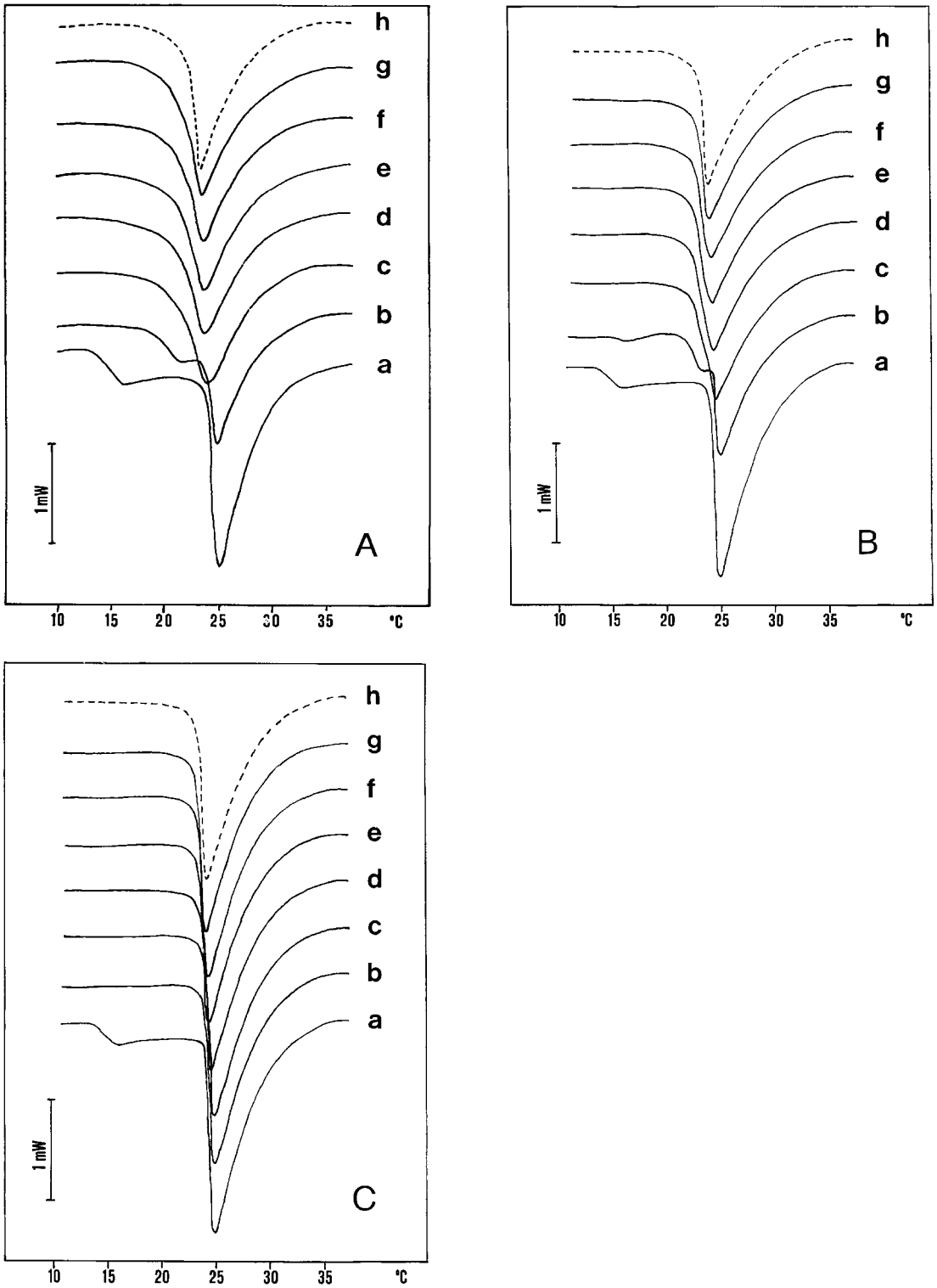

FIGURE 4. Differential scanning calorimetry heating curves of empty DMPC multilamellar vesicles alone (curves a) or left in the presence of equimolar DMPC multilamellar vesicles loaded with pyrene (A), benzo[*a*]pyrene (B), or anthracene (C) at a 0.12 molar fraction for increasing incubation times (curves b−g). Curves h represent the effect of the 0.06 molar fraction of PAH on the MLV, considered as the effect to be reached if the PAH was transferred from loaded to empty vesicles so to obtain an average amount of 0.06 in molar fraction.

molar fraction of all compounds. It is evident that the experimental values, when the transfer and interaction of PAHs present in the liposomial suspension with empty MLV dispersion occur, tend to the values representing the maximal interaction between compounds and DMPC vesicles. These values are obtained from a fixed molar fraction (0.06) prepared in the organic phase and are reported in Figure 5 by empty signs (as values at t_{inf}).

The transfer rate depends on the kind of examined molecule; in fact, pyrene (curve a″) is transferred faster than benzo[*a*]pyrene (curve b″) and anthracene (curve c″); such a intermembrane transfer seems fast during the first 2 h, and then the kinetics are almost saturated and seem to proceed only to permit the PAHs to diffuse through the interbilayer aqueous space reaching the equilibrium in the lamellar distribution, as observed in the preparation in organic

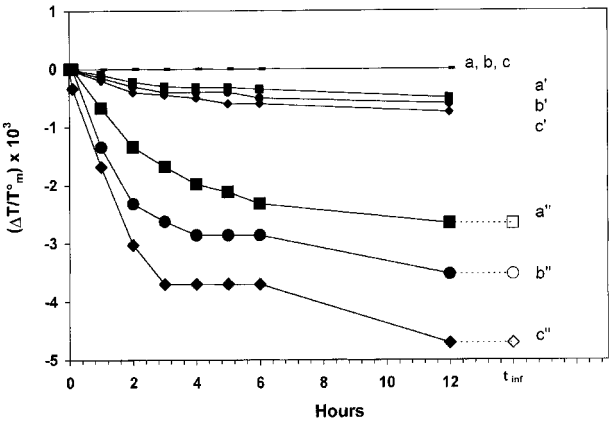

FIGURE 5. Transitional temperature variations ($\Delta T/T_m^{\circ}$), for increasing incubation times, of (1) empty DMPC multilamellar vesicles left in the presence of equimolar DMPC multilamellar vesicles loaded with pyrene (a″), benzo[*a*]pyrene (b″), or anthracene (c″) at a fixed molar fraction (0.12); (2) empty DMPC multilamellar vesicles left in the presence of pyrene (a′), benzo[*a*]pyrene (b′), or anthracene (c′) adsorbed on silica gel equivalent to a fixed molar fraction (0.06); (3) empty DMPC multilamellar vesicles left in the presence of pyrene (a), benzo[*a*]pyrene (b), or anthracene (c) as solid powdered compound so to have a fixed molar fraction (0.06). The t_{inf} values represent the effect exerted by 0.06 molar fraction of PAH on MLV, obtained starting from organic solvent solutions of lipids, to be considered as the maximum interaction between the compound and vesicles.

solvents. The differences in the rate can also be justified by considering the different aqueous solubility of the examined compounds (benzo[*a*]pyrene and pyrene are slightly more soluble than anthracene (*39*)), causing the first peak separation for the more soluble compounds.

The suggestion that the absorption process of PAHs through cell membrane is essentially mediated by a lipophilic medium is supported by the experimental results obtained by leaving in contact an exactly weighed amount (to obtain a molar fraction of 0.06) of pure solid PAHs or adsorbed on Silica gel with DMPC−MLV aqueous dispersion, inside the calorimetric DSC pan. With the submission of these samples to alternating calorimetric scans and incubation periods of 1 h at 37 °C and the comparison of the eventual calorimetric effect on model membrane with those obtained for the preparation (0.06 molar fraction) of vesicles in organic solvent, interesting considerations can be drawn. This comparison is essential to understand a PAHs ability to pass through the aqueous medium, to reach and interact with membrane models, and, at the end, to permeate the lipid layer (*24*−*26*). The calorimetric curves (curves not reported) show that all of the compounds are not able to modify the calorimetric peak shape of the pure DMPC, suggesting that they are unable to pass through the aqueous medium, even if left to incubate, at a temperature higher than the transitional temperature of DMPC, for several hours. Because of their low aqueous solubility, they are not able to reach the liposome surface and interact with the lipid layer, and consequently, no decrease in the transitional temperature of DMPC multilamellar vesicles was observed. Figure 5 clearly shows that solid PAHs (curves a, b, and c) and, to a lesser extent, the adsorbed compounds (curves a′, b′, c′) did not to pass through the aqueous medium. The small effect reported for the PAHs compounds released by the silica gel substrate can be explained both kinetically, by their presence as molecularly dispersed compounds on the silica gel substrate which offers a greater surface area with respect to the solid PAH, and thermodynamically, by the change in the equilibrium concentration of PAHs in water in contact with the solid comparated to adsorbed PAHs. Probably, under such conditions, PAHs can solubilize slightly in the aqueous medium and can be caught by model membranes, but it is interesting that the enormous increase of the exposed surface of the soil surrogate is not sufficient to permit to PAHs to be favorably absorbed by model membranes.

Several considerations can be drawn by the reported results, relevant to the possibility that PAHs could be absorbed by human cells: (a) PAHs are able to interact with model membranes as demonstrated by a shift in transitional temperature of model membranes; (b) PAHs were unable to migrate through an aqueous medium to reach biological membranes; and consequently, (c) our simple kinetic experiments demonstrate that the examined PAHs can be transferred from loaded vesicles to empty vesicles, suggesting that their absorption is favored by lipophilic agents; others mechanisms, for instance, transfer mediated by fusion of MLV, were demonstrated to be impossible (*40*).

The obtained results can also be read considering both the differences in molecular structure (sterically larger molecules better fluidify the lipid bilayer than the small ones) and solubility in the membranes. Both parameters affect the shift of lipid-phase transition temperature and the related enthalpy change (*27*, *28*). The interaction might be of relevance for PAHs carcinogenic and mutagenic properties, even if such interaction is not caused by a simple passive transport through biological membranes but they can be correlated with other mechanisms of action caused by the transfer from a lipophilic medium to a biological membrane. Finally, we have shown the importance of the calorimetric technique to detect indirectly transport through biological membranes to access the factors influencing the absorption a substance by biological membranes.

Acknowledgments

This work was supported by MURST, Cluster Project C11 No. 25, Toxicological Impact of power production plants.

Literature Cited

(1) Librando, V.; Liberatori, A.; Fazzino, D. A. *Polycyclic Aromat. Compd.* **1992**, *3*, 587−594.
(2) Librando, V.; Fazzino, D. S. *Chemosphere* **1993**, *27*, 1649−1654.
(3) Librando, V.; D'Arrigo, G.; Spampinato, D. *Analusis* **1994**, *22*, 340−342.
(4) Gerde, P.; Muggenburg, B. A.; Scott, G. G.; Lewis, J. L.; Pyon, K. H.; Dahl, A. R. *Carcinogenesis* **1998**, *19*, 493−500.
(5) Sartorelli, P.; Cenni, A.; Matteucci, G.; Montomoli, L.; Novelli, M. T.; Palmi, S. *Int. Arch. Occup. Environ. Health* **1999**, *72*, 528−532.
(6) Rummel, A. M.; Trosko, J. E.; Wilson, M. R.; Upham, B. L. *Toxicol. Sci.* **1999**, *49*, 232−240.
(7) Bommel, H.; Li-Weber, M.; Serfling, E.; Duschl, A. *J. Allergy Clin. Immunol.* **2000**, *105*, 796−802.
(8) Covance Laboratories Ltd., Harrogate, North Yorks, *Mutagenesis* **1998**, *13*, 515−518.
(9) Davila, D. R.; Lane, J. L.; Lauer, F. T.; Burchiel, S. W. *J Toxicol. Environ. Health, Part A* **1999**, *56*, 249−261.
(10) Jett, D. A.; Navoa, R. V.; Lyons, M. A. *Toxicol. Lett.* **1999**, *105*, 223−229.
(11) Inczefi-Gonda, A. *Acta Biol. Hung.* **1999**, *50*, 355−361.
(12) Brooks, R. A.; Gooderham, N. J.; Edwards, R. J.; Boobis, A. R.; Winton, D. J. *Carcinogenesis* **1999**, *20*, 109−114.
(13) McCloskey, J. T.; Oris, J. T. *Aquat. Toxicol.* **1993**, *24*, 207−217.
(14) Choi, J.; Oris, J. T. *Environ. Toxicol. Chem.* **2000**, *19*, 2699−2706.
(15) Marwood, C. A.; Smith, R. E.; Solomon, K. R.; Charlton, M. N.; Greenberg, B. M. *Ecotoxicol. Environ Saf.* **1999**, *44*, 322−327.
(16) Heo, Y.; Saxon, A.; Hankinson, O. *Toxicology* **2001**, *159*, 143−158.
(17) Mabrey-Gaud, S. Differential scanning calorimetry of liposomes. In *Liposomes: from physical structure to therapeutic applications*; Knight, Ed.; Elsevier/North-Holland Biomedical Press: Amsterdam, The Netherlands, 1981; pp 105−138.
(18) Silvius, J. R. *Chem. Phys. Lipids* **1991**, *57*, 241−252.
(19) Bach, D. Calorimetric studies of model and natural biomembranes. In *Biomembrane structure and function*; Chapman, D., Ed.; MacMillan Press: London, U.K., 1994; pp 1−41.

(20) Huang, C.; Li, S. *Biochim. Biophys. Acta* **1999**, *1422*, 273−307 and references therein.
(21) Marsh, D. In *Handbook of Nonmedical Applications of Liposomes*; Barenholz, Y., Lasic, D. D., Eds.; CRC Press: Boca Raton, FL, 1996; Vol. II, pp 21−49.
(22) Jain, M. K.; Wu, N. M. *J. Membr. Biol.* **1977**, *34*, 151−201.
(23) Jain, M. K. In *Introduction to biological membranes*; Jain, M. K., Ed.; John Wiley and Sons: New York, 1988; pp 122−165 and references therein.
(24) Raudino, A.; Castelli, F. *J. Colloid Interface Sci.* **1998**, *200*, 52−58.
(25) Castelli, F.; Caruso, S.; Giuffrida, N. *Thermochim. Acta* **1999**, *327*, 125−131.
(26) Castelli, F.; Uccella, N.; Saija, A.; Trombetta, D. *J. Agric. Food Chem.* **1999**, *47*, 991−995.
(27) Castelli, F.; Giuffrida, N.; Tringali, C.; Ruberto, G. *J. Agric. Food Chem.* **2000**, *48*, 4123−4127.
(28) Tenchov, B. *Chem. Phys. Lipids* **1991**, *57*, 165−177.
(29) Lohner, K. *Chem. Phys. Lipids* **1991**, *57*, 341−362.
(30) Castelli, F.; Pitarresi, G.; Giammona, G. *Biomaterials* **2000**, *21*, 821−833.
(31) Castelli, F.; Giunchedi, P.; La Camera, O.; Conte, U. *Drug Delivery* **2000**, *7*, 153−160.
(32) Raudino, A.; Castelli, F. In *Encyclopedia Of Colloid and Interface Science*; Hubbard, A., Ed.; M. Dekker Inc.: New York, 2002; pp 5441−5455.
(33) Rouser, G.; Fleicher, S.; Yamamoto, A. *Lipids* **1970**, *5*, 494−496.
(34) Lee, A. G. *Biochim. Biophys. Acta* **1977**, *472*, 285−344.
(35) Mouritsen, O. G.; Bloom, M. *Biophys. J.* **1984**, *46*, 141−153.
(36) Cevc, G.; Marsh, D. In *Phospholipid Bilayers Physical Principles and Models*; Bittar, E. E., Ed.; Wiley and Sons: New York, 1987; Vol. 5, p 441.
(37) Jorgensen, K.; Ipsen, J. H.; Mouritsen, O. G.; Bennet, D.; Zuckermann, M. J. *Biochim. Biophys. Acta* **1991**, *1062*, 227−238.
(38) Castelli, F.; Conti, B.; Maccarrone, D. E.; Conte, U.; Puglisi, G. *Int. J. Pharm.* **1998**, *176*, 85−98.
(39) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. *Environmental Organic Chemistry*; Wiley-Interscience: New York, 1993.
(40) Duzgunes, N.; Wilschut, J.; Papahadjopoulos, D. In *Physical Methods on Biological Membranes and their Model Systems*; Conti, F., Blumberg, W. E., De Gier, J., Pocchiari, F., Eds.; Plenum Press Publishing Corp.: New York, 1985; pp 193−218.

Received for review October 10, 2001. Revised manuscript received March 22, 2002. Accepted April 11, 2002.

ES010260W