HHS Public Access

Author manuscript

Nature. Author manuscript; available in PMC 2019 December 14.

Published in final edited form as:

Nature. 1978 October 05; 275(5679): 446–448. doi:10.1038/275446a0.

Asbestos-mediated membrane uptake of benzo[*a*]pyrene observed by fluorescence spectroscopy

J. R. LAKOWICZ, **J. L. HYLDEN**

Freshwater Biological Institute and Department of Biochemistry, University of Minnesota, County Roads 15 & 19, PO Box 100, Navarre, Minnesota 55392

EPIDEMIOLOGICAL evidence indicates that particulate matter, such as asbestos, can increase the health risk from exposure to chemical carcinogens. For example, asbestos insulation workers show an eightfold increased disposition towards lung cancer; non-smokers had only a slightly increased risk of lung cancer, whereas the smokers had a 92-fold increased risk[1,2]. These data indicate that 90% of the asbestos related cancers are the result of the co-carcinogenic effects of cigarette smoking and the inhalation of asbestos fibres. We have now examined one possible mechanism of co-carcinogenesis—the ability of particulate matter to increase the rate of cellular uptake of benzo[*a*]pyrene (BP). We used fluorescence spectroscopy to investigate the ability of two particulates, silica and asbestos, to adsorb BP, and to deliver the carcinogen to model membranes. The surface area of the asbestos sample was 60-fold less than that of our silica sample. Surprisingly the asbestos sample showed a greater ability to adsorb BP in a monomeric state than silica. Most importantly BP which is adsorbed to asbestos is more rapidly transported into membranes than BP which is adsorbed to silica. Thus increased cellular exposure to carcinogens, resulting from the presence of particulates which can adsorb and deliver these carcinogens to cells, may be involved in the process of co-carcinogenesis.

Figure 1 shows fluorescence emission spectra of BP in dilute benzene solution, and of a suspension of BP crystals in aqueous buffer. A structured emission is observed from BP dissolved in benzene, whereas the suspension of BP crystals yields a broad structureless emission at longer wavelengths. This latter emission results from formation of an excited state charge-transfer complex between two or more adjacent BP molecules[3] and is called the excimer emission. The fluorescence emission spectra may therefore be used to reveal the proximity of BP molecules to one another. Also shown in Fig. 1 is the fluorescence emission spectrum of BP which is partitioned into lipid bilayers after uptake from asbestos fibres. Only the structured emission is observed which is indicative of widely dispersed BP molecules.

Figure 2 shows the fluorescence emission spectra of BP when present as microcrystals which are suspended in aqueous buffer, and when the BP is adsorbed to silica or the amosite, which is the form of asbestos used in our studies (see Table 1 legend for experimental details). The emission spectra of the microcrystals and of the silica-adsorbed BP are similar, indicating the presence of BP microcrystals on the silica surface, or the presence of BP crystals which did not adsorb to silica during the solvent evaporation process. The emission spectra of the asbestos-adsorbed BP is dominated by the structured emission, indicating the

presence of monomeric adsorbed BP. The superior ability of amosite to disperse BP is especially surprising when the surface areas of these two particulates are compared. As determined by nitrogen adsorption these surface areas are 5.7 and 381 $m^2\ g^{-1}$ for asbestos and silica, respectively[4–6].

The fluorescence emission spectra of BP are sensitive to both the weight percentage of carcinogen which is on the particulate and to the age of the samples. Over a period of weeks after preparation the emission spectra of BP on the silica samples becomes more structured and shows less excimer emission. We do not know what factors control this process, but temperature of sample storage and the presence of residual solvent do not seem to affect the rate of sample ageing. Less significant changes occur with asbestos-adsorbed BP. Irrespective of these considerations we consistently observed more BP excimer emission from the silica than from the asbestos samples. These observations include BP-to-particle weight ratios ranging from 3 to 0.1 mg BP per gram of particulate. For both particulates the excimer emission is less pronounced at the lower weight ratios (Table 1).

Emission spectra of aqueous suspensions of particles which contain BP retain a constant intensity and shape over a period of hours. On addition of phospholipid vesicles, an increase in fluorescence intensity occurs at 405 nm. Simultaneously the excimer emission disappears. After complete transfer of BP to the lipid bilayers the emission spectra of all samples listed in Table 1 become identical to that of BP in lipid bilayers (Fig. 1). Thus, significant decomposition of BP on the surface of the particles did not occur.

BP is only slightly soluble in water, so it seems likely that BP crystals will have a cellular availability which is limited by its rate of solubilisation in the aqueous phase. A particulate which adsorbs and disperses BP in a monomeric state could therefore facilitate BP solubilisation and hence increase cellular availability, The transfer of BP from the particulate's surface to the lipid bilayer is conveniently quantitated by the increase in fluorescence intensity at 405 nm. The percentage of BP transferred is calculated from

$$\%\ \text{BP transferred to membrane} = \frac{I(t) - I_0}{I_\infty - I_0}$$

where I_0 is the fluorescence intensities at 405 nm before addition of membranes, I_∞ is the intensity after complete transfer, and $I(t)$ the intensity at time t.

Figure 3 compares the membrane uptake rate of BP from the three states. As a model membrane we used single bilayer vesicles of dipalmitoyl-L-α-phosphatidylcholine[7,8]. Asbestos-adsorbed BP shows the most rapid rate of membrane uptake, and the microcrystals of BP the slowest rate. Table 1 lists the time required for 50% transfer of BP for particulate samples with a range of BP-to-particulate weight ratios. Asbestos is more effective than silica at all weight ratios, with the most dramatic effect being observed at the lower ratios. Asbestos or silica, when added to preformed microcrystals of BP in buffer, did not increase the rate of BP membrane uptake or alter the shape of the fluorescence emission spectra of the suspended microcrystals. Therefore, only BP which is adsorbed to the particles displays

an increased rate of membrane uptake. These rates increased as surface density of BP on the particulates decreased.

Our results clearly demonstrate that a particulate with a known co-carcinogenic effect is also effective in the dispersal and transport of benzo[*a*]pyrene. This observation may partially explain the co-carcinogenic effects of particulates and polynuclear aromatic hydrocarbons[9–11]. Presumably a carcinogen must enter a cell before transformation, and such entry must involve transport of the polynuclear aromatic hydrocarbon across the plasma membrane. The lungs have mechanisms whereby particulates are cleared, these being phagocytosis by the lung macrophages and upward cilliary action followed by ingestion[12]. Particulate matter which increases the rate of carcinogen transport could therefore increase the effective dose of carcinogen before clearance by these protective mechanisms. This speculation is supported by observations which indicate that soot particles which have been isolated from human lungs no longer contain BP[13].

Particle-enhanced transport of carcinogens is unlikely to be the only mechanism of co-carcinogenesis. Studies with experimental animals have shown that the inhaled particulate haematite is co-carcinogenic with the injected systemic carcinogen diethylnitrosamine[14]. The tumours are localised in the lungs, which are the sites of particle deposition. However, particulate matter is unlikely to facilitate carcinogen uptake in these conditions where exposure to particulates and carcinogens is by different routes of administration.

Epidemiological studies have correlated human exposure to asbestos with the incidence of mesothelioma, but this cancer does not seem to be correlated with cigarette smoking[15]. Particulates such as fibrous glass[16], asbestos[10,11] and many other types of foreign bodies[17] seem to be carcinogenic without deliberate exposure to chemical carcinogens. In fact, most types of cellular trauma and scarification will predispose the damaged areas to neoplastic transformation[18,19]. Therefore it is clear that mechanisms other than particle enhanced transport are operative in carcinogenesis. The fluorescence methodology described here is useful with other polynuclear aromatic hydrocarbons[6] and with nitrogen heterocyclic compounds[20]. The ability to quantitate the delivery of carcinogens to cells should aid in elucidating the multiple etiology of carcinogenesis.

Acknowledgments

We thank the Freshwater Biological Research Foundation and especially its founder, Mr Richard Gray, Sr for assistance. This work was supported by the American Cancer Society (grant BC-261) and NIH (1R01-ES-1283). J.R.L. is an Established Investigator of the American Heart Association.

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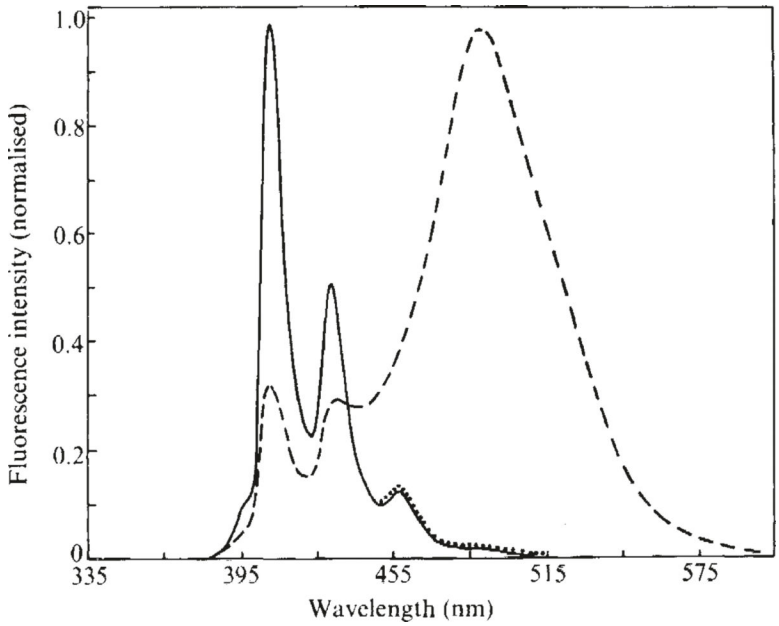

Fig. 1.

Fluorescence emission spectra of benzo[*a*]pyrene. Emission spectra are shown for BP in benzene (—), and as a suspension of crystals in aqueous buffer (- - -). After addition of DPPC vesicles to asbestos containing adsorbed BP (• • •) the emission spectra closely resembles that of BP in benzene.

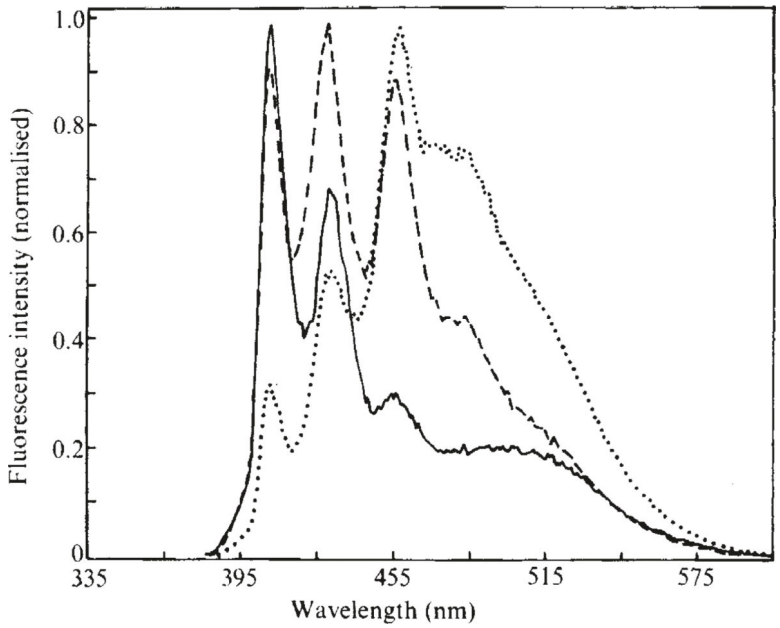

Fig. 2.

Fluorescence emission spectra of benzo[*a*]pyrene. Normalised emission spectra for BP microcrystals (• • •), and for BP adsorbed to 8.6 mg silica (- - -) and 9.5 mg asbestos (——). In each case the sample was suspended in 10 ml of buffer, and contained 5 μg BP.

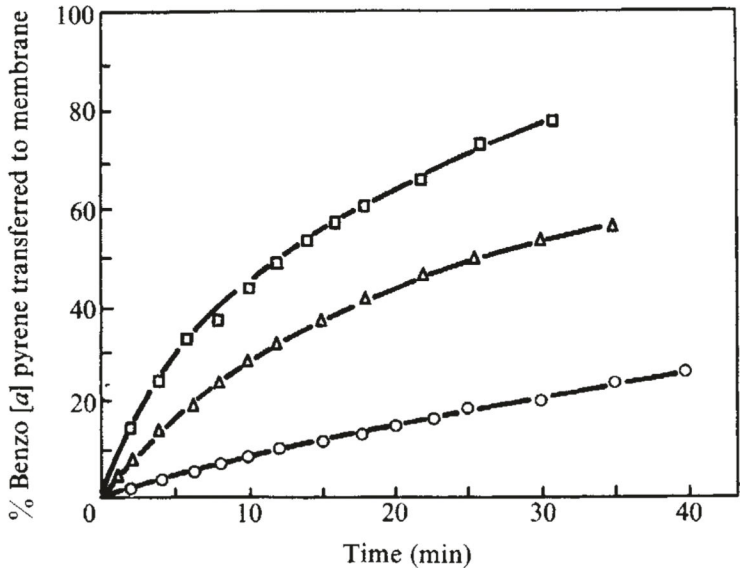

Fig. 3.

Rate of benzo[*a*]pyrene uptake in to lipid bilayers. The % of benzo[*a*]pyrene transferred to DPPC vesicles is shown for microcrystals of BP (O), silica adsorbed BP (Δ), and amosite adsorbed BP (□).

Table. 1

Benzo[*a*]pyrene uptake by phospholipid vesicles from particulate adsorbed and microcrystalline states

	Time for 50% uptake (min)		(Monomer emission)† (Total emission)	
mg BP per g particulate	Silica	Asbestos	Silica	Asbestos
0.1	4.5	3	0.32	0.34
0.3	24	6.5	0.24	0.34
1.0	30	12	0.19	0.28
3.0	34	17	0.23	0.22

BP Microcrystals	Time for 15% uptake (min)	(Monomer emission) (Total emission)
No particles	23	0.17
+ Amosite*	24	0.21
+ Silica*	24	0.19

Benzo[a]pyrene (BP) 99 + % purity was from Aldrich lot 101847 used without further purification. BP concentrations were obtained using a molar extinction coefficient of 57,500 M^{-1} cm^{-1} at 299.4 nm. Silica was obtained from preparative thin-layer chromatographic plates, 20×20 cm, 1,000 μm thick (Analabs). The surface area of this sample, as determined by nitrogen adsorption, was 381 m^2 g^{-1}. The particle size distribution is heterogeneous, with most particles being between 1.5 and 15 μm in diameter, and an average size of about 3 (μm (ref 6), Amosite served as reference asbestos sample (International Union Against Cancer, Johannesburg). The surface area determined by nitrogen adsorption is 5.6 m^2 g^{-1} The particle size distribution is heterogeneous, ranging from 1.5 to 10 μm, with the average size 2 μm. For more detailed information on size and chemical composition see ref. 5. Particulate samples containing BP were prepared by mixing the particulate with a benzene solution of BP, followed by evaporation of the benzene under reduced pressure. 0.1 to 3.0 mg of BP was added for each g particulate. The volume of benzene added was always adequate to wet the entire particulate sample, typically 5 or 6 ml of benzene per g of particulate. The sample was rotated continuously during the benzene evaporation. After the sample appeared dry it was kept under vacuum for 30–45 min at 85 °C. At the higher weight ratios BP crystals could be seen on the walls of the glass vessel. Any material which clung to the walls was discarded. After removal from the vessel the particulates were stored in the dark under an argon atmosphere. The amount of BP present on the particles was determined by solvent extraction using a Sohlex extractor. Typically the extraction was continued for 4.5 h (about 50 individual extractions). All extractions were done in duplicate. The fluorescence emission spectra of the extracted material were identical to that of the starting material. Thus there seemed to be no degradation of BP on this particulate which interfered with our measurements. The amounts of BP adsorbed to silica and asbestos, were found to be 85 ± 15% of the amount added. Aqueous dispersions of BP crystals were prepared by evaporation of a benzene solution of BP to dryness, addition of buffer, followed by sonication for 30 min at 40 W using a Cole-Parmer Model 8845–2 water bath-type sonicator. We will refer to this preparation as being microcrystalline. In all studies the buffer was 0.01 M Tris-HCI, 0.05 M KC1, pH = 7.5. Phospholipid vesicles of dipalmitoyl-L-α -phosphatidylcholine were prepared by ultrasonic irradiation of the lipid suspended at 10 mg ml^{-1}. Sonication was performed using a Heat Systems Model 350 for 10 min at ~50 °C. Power input was ~200 W. Vesicles were then centrifuged at 54,000 g for 60 min. All fluorescence spectral data were obtained on an SLM Instruments, computerised photon counting spectrofluorometer (excitation wavelength, 296 nm; excitation and emission band-passes 8 and 4 nm, respectively; excitation filter, Corning 7–54; emission filters, 0–52 and 2 mm of 1 M $NaNO_2$). BP uptake was measured by the intensity increase at 405 nm. The emission filters do not transmit light below 395 nm. Without these filters we do observe a background signal from the asbestos and silica. Some background signal was seen above 395 nm, typically <10% of the total intensity. These backgrounds were quantitated using particles without BP, and subtracted from the spectra shown. Spectral data were obtained using a 2 × 2 cm cuvette, 4 cm high, which was thermostated and positioned on a magnetic stirrer so that the stirring bar stayed below the light path. Stirring was required to keep the particles or crystals in suspension. The exciting beam impinged upon the sample at an angle of 20°, and the emission observed from this illuminated surface was at 90° to the exciting light. For all the BP uptake kinetics reported here we used 5 μg of BP and 10 mg of DPPC. An amount of particulate containing 5 μg BP, or 5 μg BP crystals, was suspended in 10 ml of buffer. After measurement of the initial intensity 10 mg of DPPC were added in 1 ml of buffer to initiate the reaction. All measurements were performed at 25 °C. Complete BP transfer to the bilayers was obtained by heating of the sample to 55 °C for 30 min. The final fluorescence intensity was measured after equilibration at the experimental temperature of 25 °C.

*Particles were added after formation of the microcrystals.

†This value was estimated from the ratio of area under the fluorescence emission spectrum from 380 to 440 nm to the total area from 380 to 605 nm. After complete uptake of BP by vesicles this ratio is 0.47.