FLUORESCENCE STUDIES OF BENZO-[A]-PYRENE IN LIPOSOME MEMBRANE SYSTEMS

T. Vo-Dinh, K. P. Li and J. D. Winefordner[a]

Department of Chemistry, University of Florida, Gainesville, FL 32611

Received August 23,1976

SUMMARY

This investigation concerns the change in the fluorescence properties of benzo-[a]-pyrene when added to membrane systems. The feasibility of the fluorescence quenching technique using metal ions which have specific transport properties to study diffusion processes of carcinogenic benzo-[a]-pyrene molecules in biomembrane systems is demonstrated.

INTRODUCTION

Studies of the mutagenic effect of carcinogenic compounds, such as benzo-[a]-pyrene have gained considerable attention among spectroscopists (1). However, not much information is available concerning the reactivity and dynamic behavior of these compounds in the presence of biological membranes which are one of the major cell components. A sensitive photochemical method of investigation involves the use of fluorescent probes as sensors for the distinct microenvironment found in these biological systems (2). The objective of this report was to investigate by means of the fluorescence technique the spectroscopic characteristics of carcinogenic benzo-[a]-pyrene in the presence of biomembrane systems and metal ion quenchers. We have selected phosphatidyl-serine and phosphatidyl-ethanolamine because they have structural and transport properties quite similar to those of natural membranes.

MATERIALS AND METHODS

Chemicals. Benzo-[a]-pyrene (BP) was purchased from Eastman Kodak (Rochester, NY) and used without further purification. The aqueous solution of BP was prepared by dissolving first 10 mg of BP in 1 ml of chloroform which was then added to 49 ml of distilled water.

Phosphatidyl serine (PPS) (Grand Island Biological Company) and phosphatidyl ethanolamine (PPE) (General Biochemical) were selected as membrane model system for our investigations. Derivatives of glycerol-3-phosphoric acid are one of

---

[a] Author to whom requests for reprints should be sent.

the most abundant classes of lipids found in plasma membranes. They were used without further purification. The phospholipid dispersions (100 mg PPS 3 ml H_2O; 25 mg PPE/3 ml H_2O) were prepared by sonication (five times for 6 min each) in an ice bath with a sonifier (Ultrasonic Inc., Model W185, Plainview, NY). The resulting milky dispersions were diluted to 50 ml and used for spectroscopic studies within the same day of preparation. Stock solution of copper chloride and copper acetate were prepared by using reagent grade salts.

Instrumentation. Fluorescence spectra were recorded with an Aminco Bowman spectrofluorometer - SPF - (American Instrument Company, Silver Spring, MD) which was modified and equipped with a Silicon Intensifier Target (SIT) image detector (Model 1250, Princeton Applied Research, Princeton, NJ). The detailed description of the luminescence apparatus has been previously given elsewhere (3).

RESULTS AND DISCUSSION

Fluorescence Spectra. Benzo-[a]-pyrene (BP) is very insoluble in water (a saturated solution of 10 mg in 50 ml contained also suspensions of aggregates of BP molecules). In Figure 1, curve *a* shows the fluorescence of BP in water. When phosphatidyl serine (PPS) or phosphatidyl ethanolamine (PPE) membrane solutions were added, the fluorescence intensity increased appreciably (Figure 1). This strong increase in the BP fluorescence signal indicates that a significant amount of BP molecules have interacted with the lipid molecules on the membrane. However, the interaction may not be the same as that Weber and Laurence (2) observed with 8-aniline-naphthalene (ANS) and its analogues.

In the case of ANS-type probes, increasing viscosity was presumably believed to reduce radiationless deactivation of the excited state occurring through the relative motion of the phenyl and naphthyl rings (4) and hence cause their fluorescence spectra to undergo a red shift in polar solvents. BP belongs to a different class of aromatic probes which have a more rigid structure and no highly polar groups, and therefore is expected to interact differently with membrane systems. The fluorescence spectrum of BP, although also originating from a $^1\pi\pi^*$ excited singlet, does not show any significant spectral shift, indicating a less important solvent stabilization effect. In the presence of PPS and PPE, the fluorescence of BP became slightly more structured than in pure aqueous solution. This might suggest that the membranes have offered to the BP molecules a more ordered environment than the largely random arrangement of the

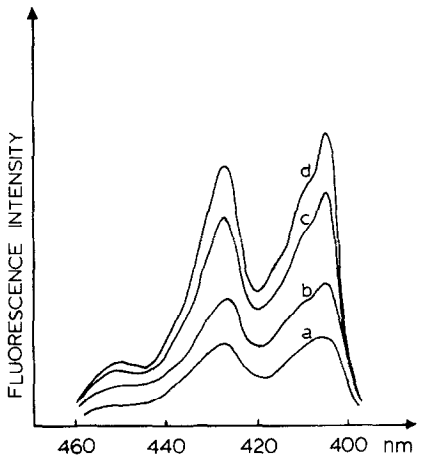

Figure 1. Fluorescence of Benzo-[a]-pyrene (BP) in the Presence of Phosphatidyl-Serine (PPS).
BP = Saturated Solution in Water
PPS = 100 mg/50 ml of Water
(*a*) BP in Aqueous Solution
(*b*) BP with PPS (5:0.05; V:V)
(*c*) BP with PPS (4.5:0.5; V:V)
(*d*) BP with PPS (2.5:2.5; V:V)

aqueous liquid state, where the fluorophores could also have a more freely rotating motion.

Quenching Studies of Benzo-[a]-pyrene by Various Metal Ions. Quenching processes of fluorescence probes by metal ions and reaction kinetics in biomembrane systems are presently a research field of increasing interest. In these investigations, the use of appropriate quenchers could provide valuable invormation about the permeability of membrane systems. Changes in the vesicle permeability are reflected in changes in the rate constant of the quenching reaction or ease of entry of the quencher to the probe which is usually incorporated inside the membrane core. In our experiment, we suggest a simple kinetic investigation of the quenching reactions using two quenchers which have similar affinity (see below) to the BP solute but different diffusion and dynamic properties with respect to the membranes. Fluorescence studies showed that Cu(II) ions efficiently quench the excited states of BP. Of particular interest are the different transport properties of Cu(II) ions when they are added to the membrane solutions

as an acetate salt or as a chloride salt. Previous studies showed that it is not the metal ion alone but the metal-anion complex as a whole that is involved in the migration and diffusion processes through lecithin membrane vesicles (5). We have here investigated fluorescence quenching studies of Cu(II) in the forms of acetate ($Cu(OAc)_2$) and chloride ($CuCl_2$) with BP in aqueous solution and in the PPS membrane system. Under steady state condition, the following relationship has been derived by Stern and Volmer to describe collisional quenching process (68), namely,

$$\frac{\phi_o}{\phi} = \frac{F_o}{F} = 1+\tau_M k_{SV}(Q) = 1+K_{SV}(Q) \qquad (1)$$

where ϕ_o and ϕ, F_o and F are the fluorescence quantum yields and intensities in the absence and presence of Q, respectively, and k_{SV} and K_{SV} are the collisional rate constant and quenching constant, respectively, and τ_M is the lifetime of the excited state, M^*.

The Stern-Volmer plots of $(\frac{F_o}{F} - 1)$ versus concentrations of Q are shown in Figure 2. In aqueous solution, the quenching constant K_{SV} of Cu(II) was higher when this metal ion was added as the chloride ($K_{SV} \cong 2.2\times10^2$ 1/mole, curve *a*, Figure 2) than as the acetate ($K_{SV} \simeq 1.3\times10^2$ 1/mole, curve *b*, Figure 2). This is probably due to the different degrees of dissociation and mobility of the metal ions in solution under different forms. In a solution of BP with PPS membrane vesicles, the quenching effect of $CuCl_2$ (curve *c*, Figure 3, $K_{SV} = 2\times10^3$ 1/mole) is much more pronounced than in aqueous solution. From equation (1), it could be deduced that the excited BP singlet has a longer lifetime when it interacts with PPS membranes. The quenching effect of $Cu(OAc)_2$ on BP fluorescence in the presence of PPS vesicles showed a time dependent behavior, the quenching effect increasing with time. This difference in the quenching behavior of $CuCl_2$ and $Cu(OAc)_2$ may be used to investigate transport process of BP in membrane systems. Our NMR data suggests that because of the difference in the degree of dissociation of the salts, the interaction of the metal species with the phosphate group of the lipid molecule will be different and so will be the transport of the metal

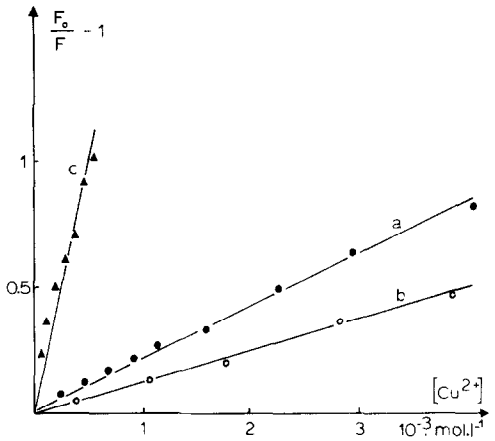

Figure 2. Quenching Effect of Benzo-[a]-pyrene (BP) by Cu^{2+}.
(a) BP with $CuCl_2$ in Aqueous Solution
(b) BP with $Cu(OAc)_2$ in Aqueous Solution
(c) BP with $CuCl_2$ in PPS Membrane Solution

ion through the vesicle membrane (5). For this reason, in our experiments, the time dependence of the $Cu(OAc)_2$ quenching effect implies that it is the undissociated Cu-anion complexes which diffuse through the membrane bilayer and get into the inside of the vesicle and interact with BP present there. The slow rate of diffusion of $Cu(OAc)_2$ caused the time dependent quenching behavior. However, free Cu(II) from $CuCl_2$, stays mainly in the outside of the vesicles, and hence quench only the BP molecules which are in the outside aqueous phase or which are on the outer layer of the PPS membrane. $Cu(OAc)_2$ can diffuse into the inside of the vesicle and hence can quench ( ) the BP molecules which are soluble in the lipid phase of the vesicle, or (ii) the BP molecules which are equilibriated in the aqueous phase inside the vesicles or both. For further investigations, we believe that the use of these selective quenchers with BP probes incubated exclusively inside or outside the vesicles would provide an effective technique to obtain valuable data for the study of toxic metal ions diffusion as well as for carcinogenesis research.

ACKNOWLEDGMENT

This work supported by NIH-GM-11373-13 and by (KPL) ACS-PRF 2896-G2 and Research Corporation.

REFERENCES

1. Sims, P. and Grover, P.L., (1974) Adv. in Cancer Res., 20, 165-275.
2. Weber, G. and Laurence, D.J.R., (1954) Biochem. J., 51, xxxi.
3. Vo-Dinh, T., Johnson, D.J. and Winefordner, J.D., submitted for publication.
4. Oster, G. and Nishijima, Y., (1956) J. Am. Chem. Soc., 78, 1581-1584.
5. Li, K.P. and Natusch, D.F.S., to be published.
6. Mataga, N. and Kubota, T., (1970) "Molecular Interactions and Electronic Spectra", Marcel Dekker Inc., New York, p. 461.