*Photochemistry and Photobiology* Vol. 37, No. 2, pp. 149–153, 1983
Printed in Great Britain. 

0031-8655/83/020149–15$03.00/0


# REMARKABLE INHIBITION OF OXYGEN QUENCHING OF PHOSPHORESCENCE BY COMPLEXATION WITH CYCLODEXTRINS

NICHOLAS J. TURRO, G. SIDNEY COX and XIN LI
Chemistry Department, Columbia University, New York, NY 10027, USA

(*Received* 5 *July* 1982; *accepted* 8 *September* 1982)

**Abstract**—The phosphorescence of the 4-bromo-1-naphthoyl group is readily quenched by molecular oxygen in homogeneous solvents. However, when this lumophore is complexed with γ-cyclodextrin in aqueous solution at room temperature, its phosphorescence is observed even under 1 atm of oxygen! Phosphorescence decay data indicated that two types of probe/cyclodextrin complexes are formed with lifetimes of 600 μs and 3.5 ms. Oxygen completely quenches the fast decay, but only partially quenches the slow decay.

## INTRODUCTION

The utilization of luminescence probes as reporters of structure, environment, and dynamics of biochemical systems has matured and flourished during the past decade (Radda, 1975; Stryer, 1978). Inspection of the literature reveals, however, that for practical purposes luminescence has been synonomous with fluorescence. Although phosphorescence probes have been identified and employed in fluid solution at ambient temperatures (Bolt and Turro, 1981; Kalyanasundaran *et al.*, 1977), the high efficiency of quenching of triplet states has severely restricted the use of phosphorescence probes in biological systems. Several reports have appeared which suggest that the triplets of aromatic amino acid residues, which are buried in the globular structure of proteins, are protected from bimolecular quenching, in particular, the ubiquitous quenching by dissolved molecular oxygen (Saviotti and Galley, 1974; Domanus *et al.*, 1979). We (Turro *et al.*, 1978) and others (Humphrey-Baker *et al.*, 1978) have shown that the phosphorescence of organic molecules may be conveniently and readily observed in fluid aqueous solution when the emitting triplet is incorporated into a conventional ionic micelle, although nitrogen purging of the sample was required. More recently, we have discovered that relatively long-lived (90 ns) excimer emission is strongly protected from oxygen quenching if the emitting singlet is included in the cavity of a cyclodextrin (CD)* (Turro *et al.*, 1982a). In another study, the steady state phosphorescence of 1-chloronaphthalene, unobservable in aqueous micellar systems, could be readily observed for aqueous cyclodextrin systems, although again nitrogen purging was mandatory (Turro *et al.*, 1982b). In the latter case, it was proposed that the rate of dissociation of the cyclodextrin/phosphorescence probe complex was limiting the efficiency of $O_2$ quenching, when quenching was efficient in the aqueous phase. If this proposal is correct, then when the complex dissociates at a rate that is slower than phosphorescence, protection from aqueous quenchers may be completely avoided. In the limiting case, oxygen dissolved in the aqueous phase would not be able to quench triplets and nitrogen purging would be unnecessary for the convenient and ready observation of phosphorescence. We report here several systems involving 1-bromo-4-naphthoyl probes included in cyclodextrins, that approach this limiting case.

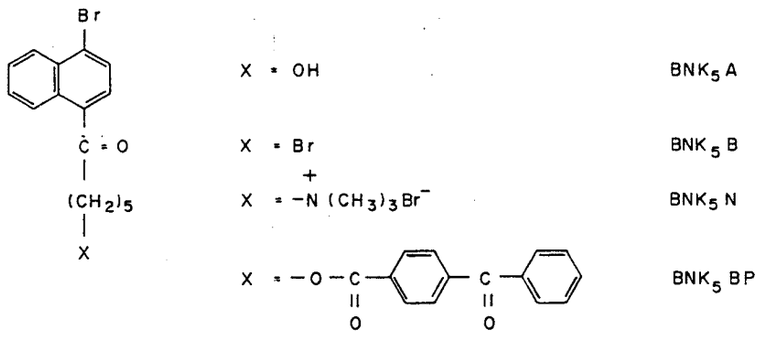
## MATERIALS AND METHODS

α, β, and γ- Cyclodextrins (Aldrich) were used as received. Hexaminocobalt (III) chloride (Alfa) was recrystallized twice from water. Water was doubly distilled. Acetonitrile was spectroquality (MCB reagents) and was used as received. The synthesis of $BNK_5N$ and $BNK_5B$ has previously been described (Bolt and Turro, 1982).

*Synthesis of $BNK_5A$.* $BNK_5B$ (10 g) was combined with silver acetate (7 g) in 120 mℓ of acetic acid and refluxed for 2 h. Water (35 mℓ) was added and the solution extracted with ether. The ether layer was washed with $NaHCO_3$ and water, and dried with anhydrous $MgSO_4$. The product ($BNK_5$ acetate) was purified by silica gel column chromatography (eluent; 5/95 ether/hexane); $^1$H-NMR (in $CDCl_3$) δ 1.67 (broad envelope, 6H), δ 3.07 (t, 2H), δ 5.10 (t, 2H), δ 8.24 (dd, 2H), δ 8.47 (dd, 2H), δ 7.56 (m, 4H). $BNK_5$ acetate (3.5 g) was dissolved in 150 mℓ of ethanol with 0.5 mℓ of sulfuric acid added. After refluxing for 2 h, the solution was evaporated to dryness. The residue was dissolved in ether, washed with $NaHCO_3$ and water, and dried with anhydrous $MgSO_4$. The product was purified by silica gel column chromatography (eluent; 75/25 ether/hexane): $^1$H-NMR (in $CDCl_3$) δ 1.67 (broad envelope, 6H), δ 3.07 (t, 2H), δ 3.60 (t, 2H), δ 8.24 (dd, 2H), δ 8.47 (dd, 2H), δ 7.56 (m, 4H).

*Synthesis of $BNK_5BP$.* 4-Benzoylbenzoic acid (2 g), 7 mℓ of $SOCl_2$ and 2 drops of DMF were stirred for 2 h. After the excess $SOCl_2$ was removed under vacuum, the product was

* *Abbreviations used:* $BNK_5A$, 5-(4-bromo-1-naphthoyl)-1-pentanol; $BNK_5B$, 1-bromo-5(4-bromo-1-naphthoyl)pentane; $BNK_5BP$, *p*-benzoyl[5-(4-bromo-1-naphthoyl)-1-pentyl]benzoate; $BNK_5N$, 5-(4-bromo-1-naphthoyl)pentyltrimethylammonium bromide; CD, cyclodextrin; NMR, nuclear magnetic resonance.




Scheme 1

recrystallized with hexanes. This product (0.75 g) and 1.2 g of $BNK_5A$ were dissolved in 100 mℓ of benzene. The mixture was refluxed for 6 h. The solution was washed with $NaHCO_3$ and $H_2O$, and dried with anhydrous $MgSO_4$. The product was purified by silica gel column chromatography (eluent; 75/25 ether/hexane). Purity was confirmed by NMR.

*Techniques*. Steady state luminescence measurements were obtained with a Perkin–Elmer Hitachi MPF-3L spectrofluorimeter. Phosphorescence lifetimes were measured as described previously using the photon counting-multichannel analyzer technique (Turro and Aikawa, 1980). The error in the lifetime measurements is 10%. Samples were Millipore filtered (0.5 μm) before use. Degassed samples were purged with nitrogen (Linde, $O_2$ free grade) for 30 min. All measurements were made at 25°C.

## RESULTS AND DISCUSSION

The phosphorescence of 1-bromo-4-naphthoyl probes ($BNK_5X$, Scheme 1) is readily observable in solution at room temperature. This emission is completely quenched by oxygen. However, in aqueous γ-CD solutions, the phosphorescence is observed even in oxygen saturated solutions (Fig. 1). The $I^0/I$ value for phosphorescence quenching by oxygen varies considerably for the 4 probes studied here (Table 1), but the shape of the phosphorescence spectra of the probes are identical for $O_2$ and $N_2$ degassed conditions. In the presence of α- and β-CD, oxygen quenches the phosphorescence, even though the probes form complexes with β-CD (Turro *et al.*, 1982c)

Phosphorescence decay curves of these probes in acetonitrile or water show single exponential decay with a lifetime of 300 μs. However, when probes $BNK_5B$, $BNK_5A$ and $BNK_5BP$ form complexes with γ-CD a more complicated decay is observed (Figs. 2 and 3). There appear to be two exponential decays: a fast decay with a lifetime of 600 μs and a slow decay with a lifetime of 3.5 ms (Table 2). This result

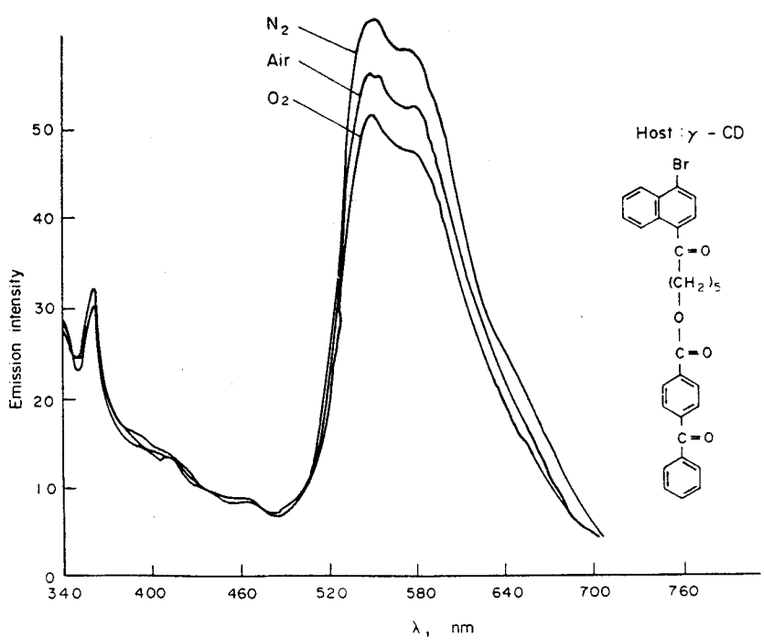



Figure 1. Phosphorescence spectra of $BNK_5BP$ in γ-CD. The effect of added $O_2$.

Table 1. Relative phosphorescence intensities of $BNK_5X$ probes in γ-CD*. The effect of $O_2$

| Probe | $O_2$ | $I^0/I$† |
|---|---|---|
| $BNK_5B$ | Air saturated | 1.11 |
| | $O_2$ saturated | 1.16 |
| $BNK_3BP$ | Air saturated | 1.26 |
| | $O_2$saturated | 1.46 |
| $BNK_5A$ | Air saturated | 20 |
| | $O_2$ saturated | 32 |
| $BNK_5N$ | Air saturated | 27 |
| | $O_2$ saturated | 50 |

* γ-Cyclodextrin concentration is 5 m*M*.
† $I^0/I$ is emission intensity under $N_2$ purged conditions ($I^0$) divided by the emission under air or $O_2$ saturated condition ($I$). Emission was measured at 570 nm.

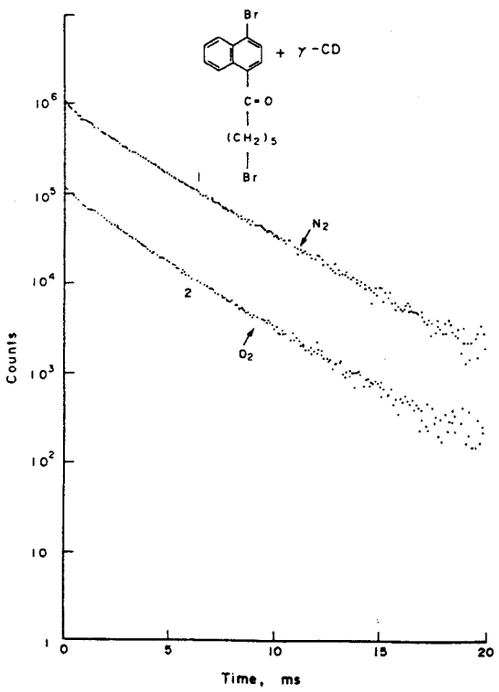



Figure 2. Typical traces of the decay of $BNK_5B$ phosphorescence in the presence of γ-CD: curve 1, $N_2$ purged; curve 2, $O_2$ saturated. Background noise was subtracted out. Curve 1 is offset by a factor of 10 counts.

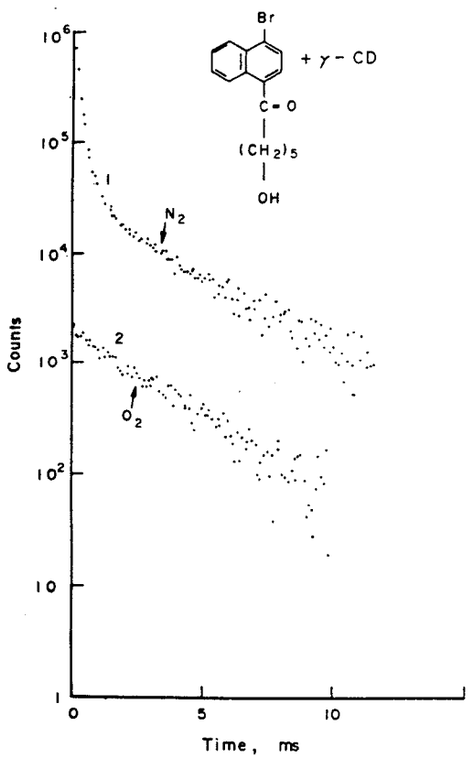



Figure 3. Typical traces of the decay of $BNK_5A$ phosphorescence in the presence of γ-CD: curve 1, $N_2$ purged; curve 2, $O_2$ saturated. Background noise was subtracted out. Curve 1 is offset by a factor of 10 counts.

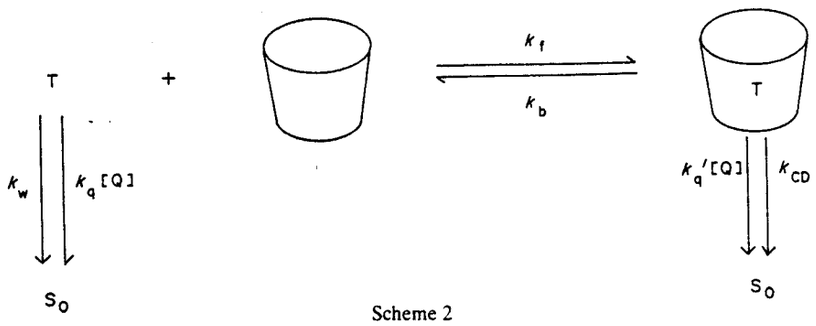
Table 2. Phosphorescence lifetimes of $BNK_5X$ probes in γ-CD*

| Probe | Lifetime (τ) | | |
|---|---|---|---|
| | Fast decay | Slow decay/$N_2$ | Slow decay/$O_2$ |
| $BNK_5BP$ | 506 μs | 3.9 ms | 3.8 ms |
| $BNK_5A$ | 645 μs | 3.3 ms | 2.5 ms |
| $BNK_5B$ | small | 3.4 ms | 2.8 ms |

* γ-Cyclodextrin concentration is 5 m*M*.

indicates that the probe exists in two environments in the presence of γ-CD. In the presence of oxygen, the fast decay is completely quenched while the slow decay is only partially quenched. The quenching of the fast decay correlates well with the $I^0/I$ values. The phosphorescence decays of $BNK_5BP$ and $BNK_5B$, which are not appreciably quenched by oxygen, consist almost entirely of the slow decay, while the decays of $BNK_5A$, which is quenched appreciably by $O_2$, consist mainly of fast decay (Figs. 2 and 3). $BNK_5N$ shows only fast decay.

To determine the nature of the complexation of these probes with γ-CD, the effect of cyclodextrin concentration on the phosphorescence of $BNK_5A$ was studied. As the concentration of γ-CD increases, the intensity of phosphorescence increases (Table 3). The intensity of phosphorescence in the presence of oxygen increases even more dramatically with γ-CD concentration such that the $I^0/I$ values decrease with increasing concentration. The lifetime for the fast decay of the phosphorescence increases with γ-CD concentration (Table 3). These results demonstrate




Scheme 2

Table 3. Phosphorescence intensity and lifetimes of $BNK_5A$: the effect of γ-CD concentration*

| [γ-CD] (m$M$) | $I_{em}$ | $I^0/I$ | τ, fast decay (μs) |
|---|---|---|---|
| 0 | 4.50 | — | 266 |
| 1 | 7.18 | >50 | 293 |
| 3 | 7.83 | >50 | 465 |
| 5 | 10.9 | 20 | 657 |
| 7 | 13.2 | 15 | 850 |
| 10 | 15.3 | 7.7 | 994 |

* $BNK_5A$ concentration 43 μ$M$.

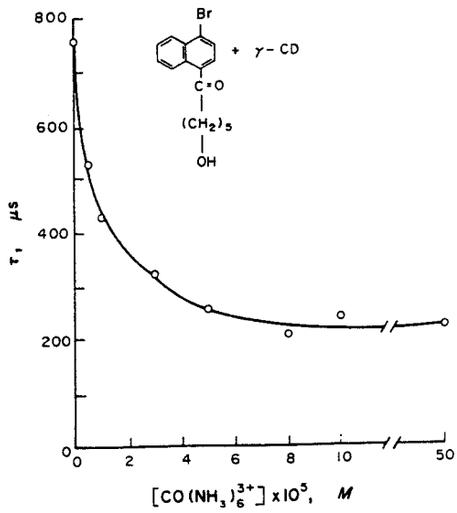



Figure 4. τ *vs* $Co(NH_3)_6^{3+}$ plot for $BNK_5A$ in γ-CD. [$BNK_5A$] = 43 μ$M$; [γ-CD] = 5.0 m$M$. Solutions are purged with nitrogen. The fast lifetimes are plotted.

that the fast decay is due to a probe/γ-CD complex and is not due to probes in the aqueous phase. One explanation for these results is that two different probe/γ-CD complexes exist, with the complexes having different rates for oxygen quenching. These results could also be explained by assuming the fast decay in the presence of cyclodextrin is a 1:1 complex, while the slow decay is a 2:1 complex containing two cyclodextrins.

The kinetic scheme for quenching of an excited state probe (T) in equilibrium with cyclodextrins has been previously described (Scheme 2) (Turro *et al.*, 1982; Almgren *et al.*, 1979). The kinetic expression for the observed rate constant for the decay of the triplet is shown in Eq. 1.

$$k_{(obs)} = \tau^{-1} = k_b + k_{CD} + k_q'[Q] - \frac{k_f k_b [CD]}{k_f[CD] + k_w + k_q[Q]} \quad (1)$$

Quenching of the $BNK_5X$ probes by oxygen can occur by two processes: (1) the probe can diffuse from the cyclodextrin and be quenched by oxygen with a rate constant $k_q$, or (2) oxygen can quench the probe/cyclodextrin complex with some rate constant $k_q'$. Since complex II (slow decay) is not appreciably quenched by oxygen, although its lifetime is long-lived, $k_q'$ for this complex must be extremely small, and $k_b$ must be less than $k_{(obs)}$. $Co(NH_3)_6^{3+}$ quenches the phosphorescence of $BNK_5X$ probes ($k_q$ = 5.3 × $10^7$ $M^{-1}$ $s^{-1}$) in aqueous solution (Bolt and Turro, 1981). The phosphorescence of $BNK_5A$ in γ-CD solution is quenched by the presence of $Co(NH_3)_6^{3+}$; however, at higher concentrations of quencher the lifetime of the probe triplet reaches a limiting value (Fig. 4). This result indicates that quenching is limited by diffusion ($k_b$) from the cyclodextrin. Also, $Co(NH_3)_6^{3+}$ quenching ($k_q'$) of the probe/cyclodextrin complex must be small. Since oxygen completely quenches the fast decay, but $Co(NH_3)_6^{3+}$ cannot, then $k_q'$ for oxygen must be larger than $k_q'$ for $Co(NH_3)_6^{3+}$.

In summary, these results indicate that the $BNK_5X$ probes form two types of complexes which have different phosphorescence lifetimes, rate constants ($k_b$) and show markedly different abilities for protecting the probes from oxygen quenching.

*Acknowledgements*—The authors thank the National Science Foundation and the Air Force Office of Scientific Research for their generous support of this work. The authors also thank Dr. I.R. Gould and Mr. Paul Hauptman for technical assistance and stimulating discussions. They acknowledge a referee's suggestion of the 2:1 complex.

## REFERENCES


Almgren, M., F. Grieser and J. K. Thomas (1979) *J. Am. Chem. Soc.* **101**, 279–291.

Bolt, J. D. and N. J. Turro (1981) *J. Phys. Chem.* **85**, 4029–4033.

Bolt, J. D. and N. J. Turro (1982) *Photochem. Photobiol.* **5**, 305–310.

Domanus, J., G. B. Strambini and W. C. Galley (1980) *Photochem. Photobiol.* **31**, 15–21.

Humphrey-Baker, R., Y. Moroi and M. Gratzel (1978) *Chem. Phys. Lett.* **58**, 207–210.

Kalyanasundaran, K., F. Grieser and J. K. Thomas (1977) *Chem. Phys. Lett.* **51**, 501–505.

Radda, G. K. (1975) *Methods Membr. Biol.* **4**, 97–188.

Saviotti, M. L. and W. C. Galley (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4154–4158.

Stryer, L. (1978) *Annu. Rev. Biochem.* **47**, 819–846.

Turro, N. J. and M. Aikawa (1980) *J. Am. Chem. Soc.* **102**, 4866–4872.

Turro, N. J., K. -C. Liu, M. -F. Chow and P. Lee (1978) *Photochem. Photobiol.* **27**, 523–529.

Turro, N. J., T. Okubo and G. C. Weed (1982a) *Photochem. Photobiol.* **35**, 325–329.

Turro, N. J., J. D. Bolt, Y. Kuroda and I. Tabushi (1982b) *Photochem. Photobiol.* **35**, 69–72.

Turro, N. J., T. Okubo and C. -J. Chung (1982c) *J. Am. Chem. Soc.* **104**, 1789–1794.