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Chiral recognition and behavior of [1,1′-binaphthalene]-2,2′-diol in aqueous solution by fluorescence spectroscopy

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Abstract

The photophysical properties of [1,1′-binaphthalene]-2,2′-diol (BINOL) have been examined with particular emphasis to the effects of chirality and chiral recognition. Few studies have specifically examined the photophysical properties of BINOL. This study was prompted by preliminary observations in our laboratory that revealed anomalously high fluorescence anisotropy values for BINOL. The analyte (BINOL) was examined in various solvents and in aqueous solutions of various pH values in order to perturb the effects of proton transfer, which was observed as a red-shift in the fluorescence emission. The anomalously high anisotropy value could not, however, be attributed to proton transfer phenomena. The anisotropy was found to be concentration dependent and was attributed to the formation of dimers or other higher order aggregates. The chiral recognition of BINOL was also examined by fluorescence anisotropy measurements and compared to that of other binaphthyl analogs.

Keywords: BOH; Binaphthol; Binol; Chiral recognition; Fluorescence anisotropy; Polarization; CD; Cyclodextrin

1. Introduction

1,1′-Binaphthalene-2,2′-diol (BINOL, binaphthol, BOH) has been widely used as the starting material for the synthesis of a great variety of molecular sensors, chiral selectors and enantioselective catalysts [1]. Structurally, binaphthol is composed of two naphthyl groups connected by a single C–C bond and exists in two stereo conformations (*R*- and *S*-BINOL) due to the steric hindrance to rotation. Study of the crystal structure of BINOL reveals that both *R*- and *S*-BINOL enantiomers are linked through hydrogen bonds via the 2,2′-hydroxyl groups [2], and the measured dihedral angles differ slightly for the two enantiomers and are reported as 101.65° and 90.58° for the *R* and *S*-enantiomers, respectively. In solution, however, both enantiomers of BINOL have dihedral angles of slightly less than 90° [3] and take a cisoid conformation [1].

Binaphthol is a luminescent molecule that exhibits a low fluorescence quantum yield. The low quantum yield has been reported as being caused by excited state rotation of the naphthol groups along C–C single bond or photo-induced proton dissociation [4]. The proton transfer of excited state 2-naphthol has been investigated in the mixture of water and methanol at varying temperatures [5]. It has been suggested that a four-water-cluster, $(H_9O_4)^+$, serves as a proton acceptor, and the rate of the proton transfer mainly depends on the formation and structure of the water cluster. It was shown that the rate constant of the proton transfer increased with increasing temperature and decreased with increasing fraction of methanol [5]. The decrease observed with increased methanol content is likely attributable to disruption of the water cluster structure and reduction in hydrogen bonding.

Although the structure of BINOL has been studied using different spectroscopic methods, few investigations have specifically examined the physicochemical properties of BINOL and binaphthyl derivatives as they relate to chiral recognition by various chiral selectors [3,4,6–8]. In terms of chiral recognition, binaphthol exhibits distinct

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behavior compared to other binaphthyl derivatives, such as 1,1′-binaphthyl-2,2′-diylhydrogen phosphate (BNP) and 1,1′-binaphthyl 2-2′-diamine (BNA). In preliminary studies in our laboratory the BINOL enantiomers were found to exhibit unexpectedly large anisotropy values in aqueous solution, which prompted a more extensive investigation of the chiral recognition and aggregation behavior of BINOL in an effort to understand the anomalous fluorescence polarization of BINOL.

2. Experimental

2.1. Material

β-Cyclodextrin was a gift from Cerestar USA Inc. (Hammond, IN). *O*,*O*′-dibenzoyl tartaric acid (DBTA), *N*-acetyl tryptophan, phenylalanine methyl ester (PAME) and mandelic acid were obtained from Sigma–Aldrich Co. (St. Louis, MO). Propranolol, tryptophan, binaphthyl-2,2′-diylhydrogen phosphate (BNP), [1,1′-binaphthalene]-2,2′-diamine (BNA), and [1,1′-binaphthalene]-2,2′-diol (BINOL) were purchased from Aldrich Chem. Co. (Milwaukee, WI). All chiral compounds were purchased as pure enantiomers and used as received. Water used in all experiments was purified by a Milli-Q system (Millipore Inc. Milford, MA) to a resistivity of at least 18 MΩ cm^{-1}. All other chemicals were used as received. The molecular micelle, poly (sodium undecanoyl-L-valinate) (PSUV) was synthesized and polymerized following procedures described in the literature [9].

2.2. Fluorescence anisotropy measurements

A modular spectrofluorometer (Photon Technology International Inc., London, Ontario, Canada) equipped with double monochromators and a photon counting PMT detector was used for all fluorescence anisotropy measurements. A Xe lamp was used as an excitation source. Measurement temperature was controlled and adjusted using a thermocirculator (NESLAB Instruments Inc., Newington, NH). Quartz cuvettes were used for all fluorescence measurements.

2.3. Solution preparation

The BINOL stock solutions used in this experiment were prepared in methanol. The solutions were prepared by allowing an aliquot of the stock solution to evaporate in a volumetric flask. The aqueous solution of the appropriate selector was then added to the flask and mixed by sonication. The vancomycin, polymer surfactant (P-L-SUV), α-, β-, and γ-CD stock solutions were prepared in phosphate buffer (50 mM, pH 6.9). Water used in all of the experiments was purified as described (vide supra).

2.4. Theory

Florescence anisotropy is a measurement based on the polarization of fluorescence emission [7,10]. When a fluorophore is excited with plane polarized light, the fluorescence emission will be polarized, the degree to which depends on the fluorescence lifetime (τ) and the rotational correlation time (θ). The rotational correlation time is in turn dependent on the molecular volume (V), temperature in K (T), and viscosity (η) of the solution. The relationship between anisotropy and these parameters is well described by the Perrin equation [10,11]:

$$\frac{r_0}{r} = 1 + \frac{\tau}{\phi} = 1 + \frac{\tau RT}{r_0 \eta V} \quad (1)$$

For a given system the measured anisotropy typically depends on molecular volume. Because the rotational correlation times for most small fluorophores are very short (typically, 80–100 ps), small molecules typically rotate significantly during their excited-state lifetimes and the anisotropy approaches zero. However, when a small fluorophore binds to a macromolecule that has a large molecular volume, the overall rotational rate will decrease, resulting in an increased anisotropy value. Thus, anisotropy measurements are sensitive to molecular size, as well as host–guest interactions and microenvironmental parameters. Additionally, if the geometry of the molecule (dihedral angle) differs in the bound form, the intrinsic anisotropy may be affected.

In terms of chiral recognition, we have recently shown that the fluorescence anisotropy is not only sensitive to host–guest interactions, but can be used to evaluate enantioselective binding [7,8,12], as shown in Eq. (2)

$$\frac{r_{\mathrm{avg},R}}{r_{\mathrm{avg},S}} = \frac{r_{\mathrm{b},R}}{r_{\mathrm{b},S}} \frac{K_R}{K_S} \frac{K_S[S]+1}{K_R[S]+1} \quad (2)$$

where $r_{\mathrm{avg},R}$ and $r_{\mathrm{avg},S}$ represents the measured anisotropy for the *R*- and *S*-enantiomers in the presence of the chiral selector, K_R and K_S the association constants, $r_{\mathrm{b},R}$ and $r_{\mathrm{b},S}$ the anisotropy of the bound species; and [*S*] is the concentration of free selector (host molecule). Eq. (2) effectively represents the effects of differential binding on the steady-state anisotropy values. Thus, it has been shown [7,8,12] that fluorescence anisotropy can be used to examine chiral discrimination between two enantiomers even though they may exhibit the same spectral features. Here, the fluorescence anisotropy and the observed fluorescence spectral shifts are used to investigate the aggregation and chiral recognition of BINOL.

3. Results and discussion

3.1. Fluorescence anisotropy of BINOL

The fluorescence anisotropy of BINOL was evaluated in various solvents (Table 1). The largest anisotropy value was observed in phosphate buffer at a pH of 6.9 (r = 0.187) while smallest was observed in a mixture of 95% hexane and 5% isopropanol (r = 0.011). It is interesting to note that the anisotropy of BINOL is unusually high in the most polar

Table 1
Anisotropy of BINOL in various solvents

No.	Solution	*r*	S.D.
1	Hexane/isopropanol (95:5)	0.012	0.001
2	Chloroform	0.026	0.001
3	Isopropanol/water (50:50)	0.074	0.001
4	Acetonitrile/water (50:50)	0.088	0.006
5	Acetonitrile/water (10:90)	0.103	0.004
6	Methanol/water (50:50)	0.123	0.005
7	Water	0.167	0.004
8	Phosphate buffer	0.187	0.003

solvents. Fig. 1 shows the anisotropy of three binaphthyl derivatives with various chiral selectors, including α-, β-, and γ-cyclodextrins, and vancomycin. In all cases examined, BINOL shows greater anisotropy values compared to the other binaphthyl analogs. The fluorescence lifetime of BINOL in an aqueous solution has been reported in the presence of permethylated β-cyclodextrin [4], where *S*-BINOL was reported to exhibit a double exponential fluorescence decay with lifetimes of 2.9 ns (95%) and 23 ns (5%), while *R*-BINOL displayed a single decay curve with a lifetime of 6.6 ns. The differences in fluorescence lifetimes can be expected to affect the steady-state anisotropy, but do not explain the high anisotropy values observed in this study.

Thus, the exceptionally high anisotropy observed for BINOL in polar solvents can be attributed to the following possible phenomena: (1) proton transfer from hydroxyl groups of BINOL to water clusters, (2) intra-molecular rotation of the two naphthalene planes along the C–C single bond, and (3) strong interactions between BINOL molecules such as the formation of dimers or larger order aggregates.

3.2. Proton transfer of BINOL

To investigate the effects of proton transfer on the anisotropy of BINOL, the pH dependence of the fluorescence spectra was examined. Fig. 2 shows the spectra of *R*-BINOL measured in phosphate buffer (50 mM) at different pH values (pH 3.2, 6.9, and 12.0). The fluorescence emission of *R*-BINOL decreases in intensity as pH increases. A significant red shift in the maximum emission was observed with

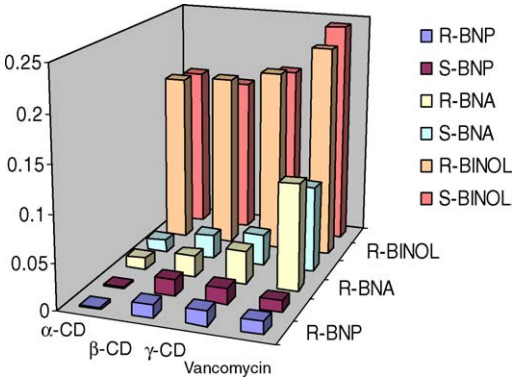

Fig. 1. Anisotropy of three binaphthyl derivatives with various selectors.

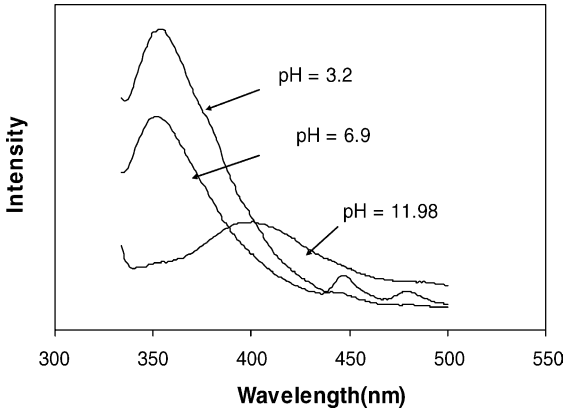

Fig. 2. Fluorescence emission spectra of BINOL at different pH.

the pH 12.0 solution (from 358 to 410 nm), which can be ascribed to the deprotonation of BINOL.

The anisotropy was also measured at an extremely low pH (1.6), at which proton transfer should be effectively eliminated. Table 2 shows the anisotropy values of BINOL obtained at pH 1.6 and 6.9, respectively. The anisotropy was measured at two different excitation wavelengths (290 and 333 nm). Only a small decrease in anisotropy is observed as the pH changes from 6.9 to 1.6. Since the proton transfer of BINOL is substantially restricted under acidic conditions (pH 1.6), the data supports the theory that the proton transfer is not the major cause of the anomalously large anisotropy values observed for BINOL.

Fig. 3 shows the fluorescence emission spectra of BINOL in water and in solvents of various polarities. An enhancement

Table 2
Anisotropy values of *R*-BINOL at various pH values

Solution pH	Excitation (nm)	*r*	SD
1.6	278	0.0780	0.0370
6.9	278	0.1165	0.0440
1.6	290	0.1958	0.0031
6.9	290	0.1711	0.0026
1.6	333	0.2418	0.0086
6.9	333	0.2546	0.0110

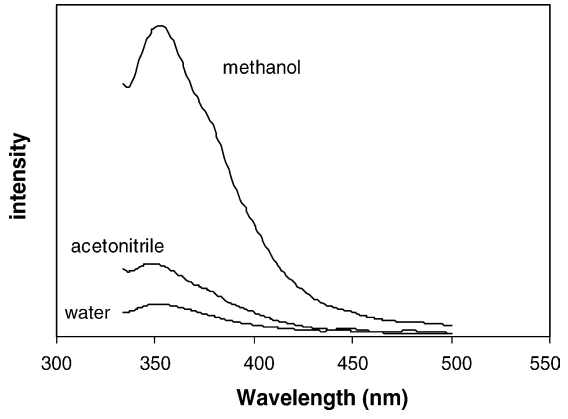

Fig. 3. Emission spectra of BINOL in various solvents.

in fluorescence intensity is observed in the less polar solvents, especially in methanol, which may be attributed to the restricted proton transfer in the methanol solution, resulting in a larger fluorescence quantum yield. This observation is in agreement with the experiments conducted with structurally related 2-naphthol [5], which indicated that the proton transfer process associated with 2-naphthol was considerably retarded by the addition of methanol. The experiment shows no apparent deprotonation in a mixture of 50% methanol and 50% water. The fluorescence anisotropy of BINOL was also measured in a mixture of methanol and water (50:50). A large anisotropy value was obtained (0.1513), suggesting that proton transfer is not responsible for the large anisotropy of BINOL.

3.3. Concentration dependence of fluorescence anisotropy

To investigate dimerization of BINOL in aqueous solutions, the concentration dependence of the anisotropy was examined. Fig. 4 shows the anisotropy of BINOL as a function of concentration. The measured anisotropy increased with increasing BINOL concentration, an initially surprising observation since the fluorescence anisotropy is fundamentally independent of concentration. Thus, the observed concentration dependence is likely a result of molecular interactions, such as hydrogen bonding and π–π stacking that could lead to the formation of dimers or higher order aggregates, the concentration of which would increase with the shift in equilibrium caused by increasing the total BINOL concentration.

Fig. 5 shows the excitation spectra of *R*-BINOL in a mixed solvent of methanol and water. Three major excitation bands are observed (278, 290 and 333 nm). Increasing the concentration of BINOL enhanced the excitation bands at 333 nm, while it decreased the intensity at 278 nm. Fig. 6 shows the fluorescence emission spectra of *R*-BINOL at various concentrations. Two emission bands are observed (300 and 362 nm) at an excitation wavelength of 278 nm. The emission intensity at 362 nm increases with increasing concentration while the intensity at 300 nm decreases accordingly. Fig. 7 shows the emission spectra of BINOL obtained at an excitation wavelength of 290 nm. A significant increase in emission intensity is observed with increasing concentration of BINOL. This enhancement of fluorescence emission is accompanied by a red shift in maximum emission (from 350 to 362 nm). The observed red shifts and intensity enhancement support the theory that BINOL exists in

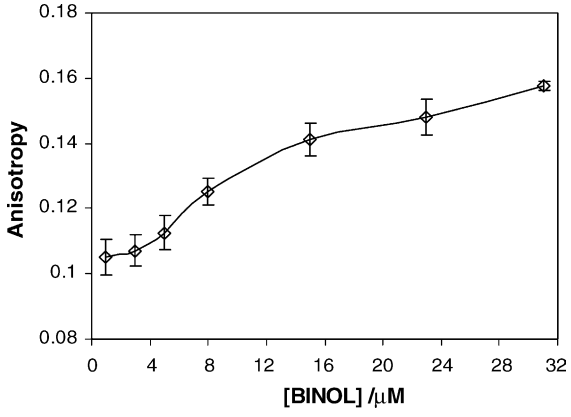

Fig. 4. Concentration dependence of anisotropy of BINOL.

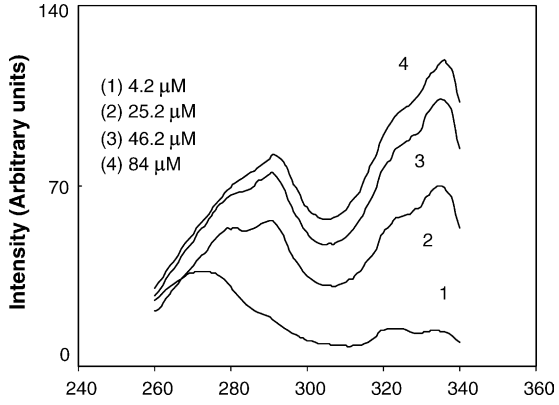

Fig. 5. Excitation spectra of BINOL in methanol/water.

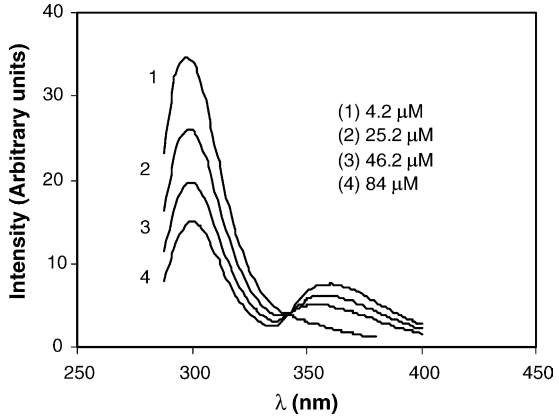

Fig. 6. Emission spectra of BINOL in methanol/water (exc. 278 nm).

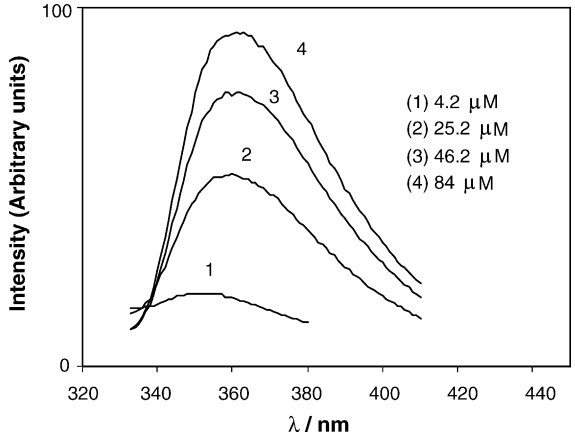

Fig. 7. Emission spectra of BINOL in a mixture of methanol/water (exc. 290 nm).

various forms (dimer, etc.) in an aqueous solution at comparatively low concentrations. The excitation and emission bands at shorter wavelengths may be assigned to free BINOL molecules while the bands at longer wavelengths may be attributed to larger spices such as dimers or larger aggregates (BO^- band ~400 nm). Fig. 8 shows the excitation anisotropy spectrum of BINOL in methanol/water (50:50) at 20 °C. As is typically observed, the anisotropy varied as a function of excitation wavelength, with maxima corresponding to the spectral features observed in the excitation spectrum.

3.4. Anisotropy of pure enantiomers and the racemate of BINOL

The spectral and anisotropy studies for BINOL are indicative of strong interactions between BINOL molecules (vide supra). It has been reported that BINOL can form dimers via hydrogen bonding in chloroform [13]. Two different dimers, homochiral, and heterochiral, were reportedly formed by single enantiomers and the racemate with equilibrium constants of 3.1 ± 1.0 and $1.3 \pm 0.5\,M^{-1}$ for the hetero- and homochiral dimer, respectively. As is shown in Table 1, BINOL has an anisotropy value ($r = 0.026$ in chloroform) that is twice as large that measured in 95/5 hexane/isopropanol solution ($r = 0.012$). A difference in anisotropy could result from the formation of dimers, however, the magnitude of anisotropy in aqueous solution is still much larger than that obtained in chloroform (Table 1). To further investigate the nature of the observed interactions, the anisotropy of BINOL was measured in both chloroform and in aqueous solution with pure enantiomers and the racemate. The results are shown in Table 3. In the both cases, the anisotropy of single enantiomers (r_R) is slightly larger than that of a mixture of *R*- and *S*-enantiomers (r_{R+S}). Fig. 9 shows the excitation and emission spectra of enantiomers and racemate of BINOL in an aqueous solution. The emission intensity of racemate is slightly higher than that of single enantiomers, which may result from the different structures of the dimers formed by single enantiomers and the racemate.

Another possible reason for the large anisotropy of BINOL is the conformation of the naphthalene planes of BINOL around the C–C single bond, which could affect the fluorescence lifetime, quantum yield, and the angle between the absorption and emission dipoles (α-angle). Additionally, it has been reported that the binaphthyl molecule can be racemized quickly via intra-molecular rotation with a half-life of 14.5 min at 50 °C [1]. However, the racemization of binaphthyls is significantly hindered when substituents are introduced at the 2,2-positions, due to restricted rotation along the intra-annular bond. BINOL bears two hydroxyl groups at 2,2′-positions and is capable of forming intra- or inter-molecule hydrogen bonding. Thus, a low racemization rate should be expected in an aqueous solution. This can also be further verified by comparing the anisotropy of BINOL measured in the presence and absence of a host molecule/receptor. For example, the anisotropy values of BINOL with β- and γ-CD are 0.1874 and 0.1988, respectively and are very close to the anisotropy measured without CDs, $r = 0.1873$. Variation in the intra-annular bond conformation should be considerably reduced by the formation of an inclusion complex between BINOL and cyclodextrins. Thus, if intra-annular bond rotation is a major cause of the increased anisotropy of BINOL, the measured anisotropy should decrease significantly upon complexation.

Table 3
Anisotropy of single and mixing BINOL enantiomers

Solution	Anisotropy	S.D.	r_R/r_{R+S}
R-BINOL in chloroform	0.0262	0.0009	1.0234
R + *S*-BINOL in chloroform	0.0256	0.0006	
R-BINOL in 50% MeOH	0.1513	0.0085	1.0265
R + *S*-BINOL in 50% MeOH	0.1474	0.0069	
R-BINOL in 50% MeOH[a]	0.1813	0.0003	1.0143
R + *S*-BINOL in 50% MeOH[a]	0.1788	0.0009	
R-BINOL in buffer	0.2199	0.0116	1.0261
R + *S*-BINOL buffer	0.2143	0.0146	

[a] Excitation 333 nm. All other data was measured at excitation 290 nm.

3.5. Study of chiral recognition for BINOL

The measured anisotropy has been shown to be a good indicator of chiral interactions and chiral selectivity [7,8,12].

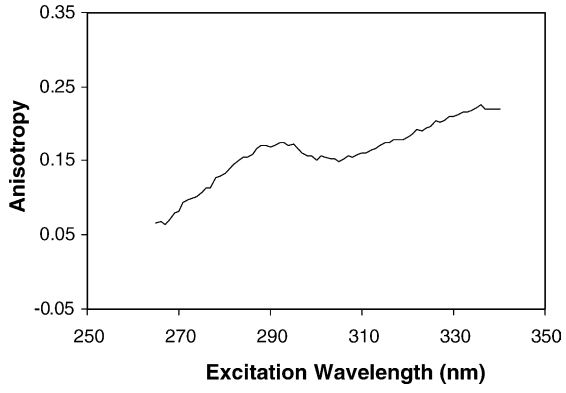

Fig. 8. Excitation anisotropy spectrum of BINOL.

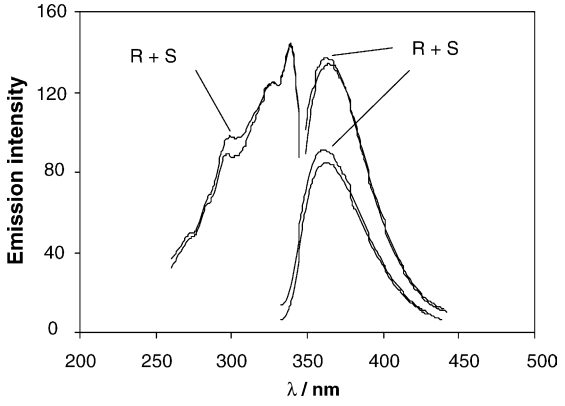

Fig. 9. Excitation and emission spectra of BINOL in a aqueous solution.

Table 4
Anisotropy data of BINOL with various chiral selectors

Selectors	r_R	S.D.	r_S	S.D.	r_R/r_S	S.D.
α-CD	0.1829	0.0033	0.178	0.0074	1.026	0.018
β-CD	0.1874	0.0009	0.1702	0.0018	1.101	0.012
γ-CD	0.1988	0.0009	0.1885	0.0025	1.055	0.017
Vancomycin	0.2297	0.0024	0.2434	0.0036	1.060[a]	0.019
PSUV in buffer	0.2204	0.0011	0.2188	0.0011	1.007	0.007
s-β-CD in buffer	0.1319	0.0027	0.1408	0.0074	1.067[a]	0.060
s-β-CD in isopropane	0.0886	0.0004	0.0900	0.0009	1.016[a]	0.012
s-β-CD in MeOH	0.1208	0.0021	0.1209	0.0014	1.001[a]	0.021

[a] r_S/r_R.

The enantiomers of BINOL displayed very large anisotropy values in the presence of chiral selectors. Theoretically, when BINOL enantiomers form complexes with chiral selectors such as cyclodextrins, photo-induced deprotonation and intra-annular rotation of naphthol moieties are highly restricted. Thus, the large anisotropy values of BINOL may be attributed to dimerization of BINOL molecules. The anisotropy ratios of *R*- and *S*-BINOL measured in the presence of various chiral selectors are shown in Table 4.

Fig. 10 compares the anisotropy of BINOL with six different selectors (including α-, β-, and γ-cyclodextrins, vancomycin, polymer micelle, and sulfated cyclodextrin). All BINOL-selector systems examined show large anisotropy values ($r > 0.1$). Fig. 11 shows the anisotropy ratios of *R*- and *S*-BINOL with the same chiral selectors. β-cyclodextrin shows the highest anisotropy ratio ($r_S/r_R = 1.1$). In comparison with other binaphthyl derivatives such as BNA and

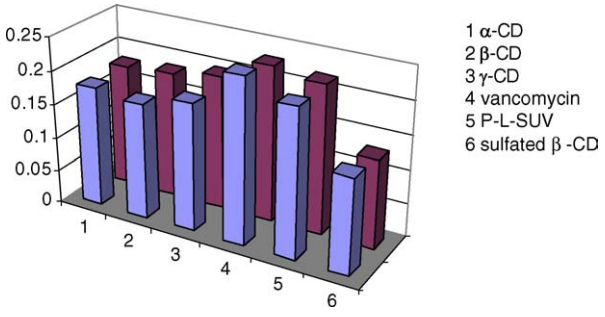

Fig. 10. Anisotropy of the *R* (■) and *S* (■) enantiomers of BINOL with various selectors.

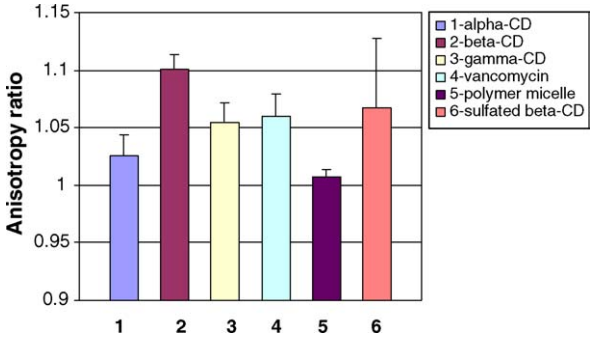

Fig. 11. Anisotropy ratio of BINOL enantiomers with various selectors.

BNP, BINOL shows a very large anisotropy value but relatively small anisotropy ratio. The chiral separation of BINOL by capillary electrophoresis with β-cyclodextrin as a chiral selector was reported [14] and no measurable chiral resolution was observed without using a dual selector system. The capillary electrophoretic studies of *R*- and *S*-BINOL was also reported using monosaccharides as chiral selectors, but no chiral resolution was observed ($\alpha = 1$) [13]. A chiral selectivity of 1.029 was reported recently using open-tubular CEC coated with an amino acid based polymer surfactant [15]. Interestingly, a much higher enantioselectivity has been achieved ($\alpha = 1.4$) in HPLC using a chiral stationary phase and a non-polar mobile phase (a high content of hexane) [14]. The chiral selectivity was found to decrease rapidly with increasing polarity of the mobile phase. The enhancement of chiral separation may result solely from partitioning effects, however, a possible explanation for the increased selectivity in hexane is that aggregate/dimer formation is suppressed. The additional equilibrium of self-association will obviously affect chiral separation of BINOL, as the host–guest interaction of the dimer will certainly differ from those of the monomer.

4. Conclusions

The molecular properties and chiral recognition of binaphthol have been investigated using fluorescence spectroscopy. A red shift in the fluorescence emission was observed with increasing pH values, which can be attributed to the deprotonated species of BINOL. BINOL exhibited large anisotropy values in solutions in which proton transfer is highly restricted, indicating that proton transfer does not explain the anomalously large anisotropy values observed for BINOL. This evidence supports the idea of dimer or aggregate formation driven by homo-inter-molecular interactions, such as hydrogen bonding or π–π stacking. Very strong evidence supporting this explanation is given by the concentration dependence of the anisotropy. Additionally, the increase in BINOL concentration resulted in spectral shifts in both excitation and emission spectra, which indicates the formation of a new species, rather than an excited-state process that would only affect the emission spectrum. Similar to the anisotropy measurements made in chloroform in which

BINOL was found to form different dimers, slight differences in anisotropy and emission spectra were also observed for single enantiomers and the racemate, possibly due to the formation of homochiral and heterochiral dimers in aqueous solutions. The chiral recognition of BINOL was investigated by fluorescence anisotropy using various chiral selectors. The anisotropy ratios (chiral recognition) of BINOL were found to be much smaller than other binaphthyl derivatives such as BNP and BNA. The minimal chiral recognition observed for BINOL may be attributed in part to the dimerization of BINOL, which would affect the interactions between BINOL and chiral selectors.

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