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Enantioselective detection of chiral phosphorescent analytes in cyclodextrin complexes

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Abstract

Inclusion complexes between camphorquinone (CQ) and cyclodextrins (CDs) in deoxygenated aqueous solutions are shown to exhibit relatively strong room temperature phosphorescence (RTP). Among the various CDs tested, α-CD showed the strongest RTP signals. Interestingly, these signals differed significantly for the two enantiomers of CQ; the phosphorescence lifetime of (+)-CQ was about four times longer than that of (−)-CQ, being 352 ± 16 and 89 ± 6 μs, respectively. This enantiomeric selectivity is attributed to a difference in dissociation rates (competing with the radiative emission process) for the diastereoisomeric inclusion complexes dealt with, which have a 2:1 stoichiometry (α-CD:CQ:α-CD). Time-resolved RTP detection using different delay times enables the determination of the two enantiomers in a mixture without involving a separation technique. The minimum detectable fraction of (+)-CQ in a 2 mM sample was 13%.

Keywords: Camphorquinone; Inclusion complex; Room temperature phosphorescence; Phosphorescence lifetime

1. Introduction

Available enantioselective detection methods, i.e. optical rotatory dispersion and circular dichroism, rely on the differential interaction of a stereoisomer with circularly polarized light. They are based on a differential measurement in the presence of a large background, which limits the sensitivity that can be achieved. Physical differences for the stereoisomers can be highlighted by turning them into diastereoisomers, but also for diastereoisomers conventional spectrochemical detection methods such as UV–vis absorption and fluorescence are usually not sufficiently selective. The determination of enantiomeric purity remains a major challenge in analytical chemistry. Most current methods are based on enantioselective separation techniques, such as chiral stationary phases in liquid chromatography and gas chromatography, and cyclodextrins (CDs) in capillary electrophoresis (CE) [1,2]. Recently, some enantioselective fluorescent sensors based on the formation of diastereoisomeric inclusion complexes with mandelic acid have been developed [3,4].

CDs are cyclic oligosaccharides, which in aqueous solutions can form cyclodextrin-analyte inclusion complexes with various types of compounds. The CDs used for analytical purpose are α-, β- and γ-CD, which contain six, seven, and eight glucose units, respectively. In recent years, derivatized CDs have also been widely used in analytical chemistry, for instance in CE [2,5].

Several reviews have discussed the spectroscopic effects of CDs and how these can be applied in analytical chemistry [6,7]. Under deoxygenated solvent conditions, CDs can enhance the room temperature phosphorescence (RTP) of various compounds such as 6-bromo-2-naphthol derivatives [8–11], polynuclear aromatic hydrocarbons [12,13], or acid–base indicators such as neutral red [14]. In the case of some exceptional, well-protected complexes strong RTP signals can even be observed without deoxygenation [15].

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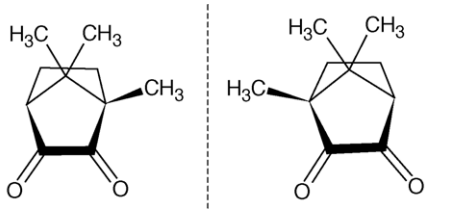

Fig. 1. Structure of the enantiomers of CQ.

Tran and Fendler [16] reported how CDs can be used to discriminate between (+)- and (−)-α-(1)-naphtylethylamine based on differences in fluorescence lifetimes. Studies using NMR spectra [17] as well as papers dealing with spectroscopic and photophysical investigations [18,19] also show the possibility of chiral discrimination by CDs for bicyclic compounds such as camphorquinone (CQ), the compound concerned with in the present paper. CQ is a chiral bicyclic 2,3-dione (see Fig. 1) and is widely used as photosensitizer for dental resin composites [20], or as photo-initiator for light-cure resin compositions [21,22] due to its photochemical reactivity upon excitation in the 400–500 nm range [23]. So far, its liquid-state room temperature phosphorescence spectra have been reported for nonpolar solvents as methylcyclohexane but not yet for aqueous CD solutions [24]; in aqueous solvents phosphorescence is hardly visible [18].

In the present paper, it will be shown that liquid-state RTP in combination with the use of appropriate CDs provides a high selectivity between the optical isomers of a phosphorescent compound such as CQ, while absorption and fluorescence hardly show any difference. The phosphorescence lifetimes observed differ strongly, so that time discrimination for selective enantiomer detection is readily performed. It should be noted that CQ is used here as a model compound; the same method is expected to be applicable to a selected group of other chiral compounds that emit RTP in the liquid state, such as α-, β-unsaturated ketones (including the well-known testosterones) and chiral binaphthyl compounds. Furthermore, application of the RTP technique is not necessarily limited to phosphorescent analytes. In a separate study, we investigated the RTP of 1-bromonaphthalene in ternary complexes with β-CD and with (+)/(−)-menthol as analyte and demonstrated that RTP lifetimes can be used to discriminate between the non-phosphorescent menthol enantiomers (see accompanying paper [15]).

2. Experimental

2.1. Chemicals and samples

(±)-Camphorquinone ((±)-CQ), carboxymethylated-β-cyclodextrin (CM-β-CD, degree of substitution (d.s.) ∼ 3), α-cyclodextrin (α-CD) and (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD, d.s. ∼ 0.6) were purchased from Fluka (Buchs, Switzerland). (1*R*)-(−)-Camphorquinone ((−)-CQ), (1*S*)-(+)-camphorquinone ((+)-CQ), β-cyclodextrin (β-CD), and γ-cyclodextrin (γ-CD) were purchased from Aldrich (Steinheim, Germany). All chemicals were used as received. Water used for the preparation of the solutions was purified through a Milli-Q system from Millipore (Bedford, MA, USA).

2.2. Instrumentation

An LS-50B luminescence spectrometer (Perkin-Elmer, Beaconsfield, UK) provided with a home-made set-up for continuous nitrogen deoxygenation of the solutions was used. For this purpose, the spectrophotometer was adapted for a special luminescence cuvette (Hellma Benelux BV, The Netherlands) with a cap, fitted with Teflon tubes, so that a low nitrogen flow through the sample could be maintained.

In order to remove interfering fluorescence from the phosphorescence spectra, a delay time of 0.10 ms was used (relatively long in order to fully reject scattered light from the pulsed excitation lamp); the gating time was typically 5.00 ms. For the lifetime measurements the gating time was fixed at 0.05 ms, while the delay time was varied from 0.10 to 1.40 ms. These values were chosen after exploratory measurements had shown that the lifetimes were in the 100–400 μs range.

2.3. Analytical procedure

Solutions of racemic CQ and separate enantiomers of CQ were prepared by dissolving in Milli-Q water, or in Milli-Q water with an appropriate amount of cyclodextrins (α-CD, β-CD, γ-CD, HP-β-CD, CM-β-CD) up to a final CQ concentration ranging from 0.5 to 5.0 mM. The CD concentrations were 10 mM for the initial comparison of the various CD's; in further studies the concentration of α-CD was varied over a 5–30 mM range and 20 mM for the lifetime measurements. In view of the photochemical reactivity of CQ, solutions were freshly prepared each day and kept in the refrigerator covered with aluminium foil until analysis. During the 10 min of nitrogen deoxygenation, which was found to be sufficient, the lamp of the spectrometer was kept off to avoid photodegradation, only to be switched on just before performing the measurements. Photodegradation was further avoided by using high spectral scan rates. Enantiomeric mixtures of CQ were made by mixing known amounts of single-enantiomeric stock solutions by weight. All data handling was done using Origin 6.1 from OriginLab Corporation.

3. Results and discussion

As expected [24], RTP of CQ in deoxygenated aqueous solution was extremely weak although not completely absent (see Fig. 2). However, in the presence of a 10 mM concentration of various cyclodextrins (α-CD, β-CD, γ-CD, HP-β-CD, CM-β-CD), an enhancement of the phosphorescence was ob-

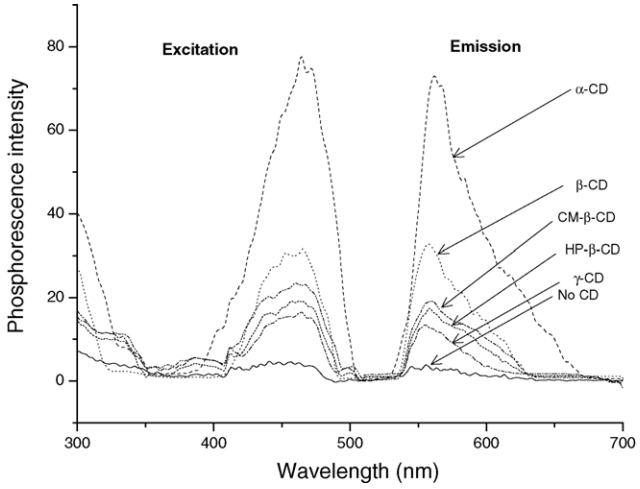

Fig. 2. Phosphorescence excitation and emission spectra of racemic CQ (2.0 mM) in aqueous solution (bottom curve) and in the presence of 10 mM of various cyclodextrins as specified in the graph. Delay time: 0.10 ms, gating time: 5.00 ms; λ_{exc} = 465 nm, λ_{em} = 562 nm; excitation and emission band widths: 10 nm.

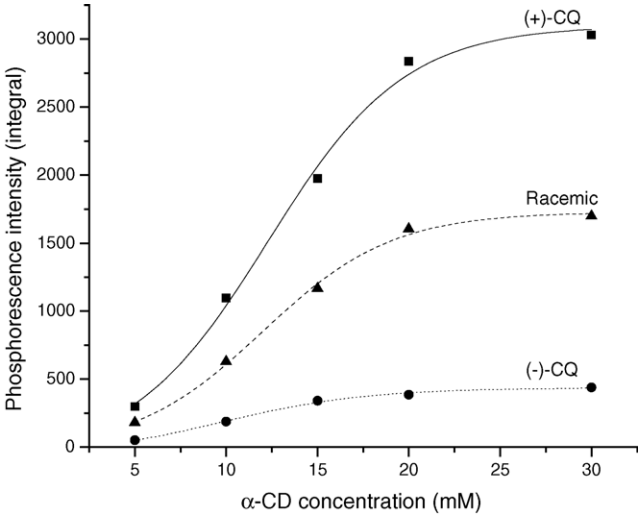

Fig. 3. Effect of α-CD concentration on phosphorescence emission of racemic CQ (▲), (+)-CQ (■), and (−)-CQ (●) (2.0 mM). Delay time: 0.10 ms, gating time: 5.00 ms; λ_{exc} = 470 nm, λ_{em} = 562 nm; excitation and emission band widths: 10 nm.

served, as shown for racemic CQ (2.0 mM) in aqueous solution. The emission maxima were slightly red shifted in the presence of cyclodextrins, as expected in view of the decrease in polarity and reorientation effects [24]. The highest phosphorescence intensity was observed in the case of α-CD. This may seem surprising, since α-CD is too small to accomodate CQ. However, it is known [18] that with α-CD 2:1 complexes are formed, whereas with the larger CDs the 1:1 complexes dominate. Presumably, the RTP intensities observed reflect the degree of protection of the CQ triplet state provided by the inclusion complex against the aqueous environment. An increase in phosphorescent lifetime (see below) also indicated that protective complexes are formed, and that the increase in intensity is not simply due to increased solubility.

Different concentrations of α-CD were added to 2 mM solutions of racemic CQ or each of its enantiomers. Fig. 3 shows that above a [α-CD]/[CQ] ratio of 10, a plateau was reached for the RTP enhancement for both enantiomers, indicating that the 2:1 complex formation is practically complete, in full agreement with the association constants reported in the literature [17,18]. Furthermore, in a 20 mM α-CD solution, the signal varied roughly linearly with the CQ concentration over the range 0.5–2.0 mM CQ (not shown). Fig. 3 also reveals that the phosphorescence intensities of the two CQ enantiomers in the α-CD inclusion complex are very different. In other words, the formation of diastereoisomeric complexes of CQ with α-CD (in agreement with the literature [17–19,25]), does allow chiral discrimination by means of RTP, without the need to involve a separation technique. The same was not found for the 1:1 CQ–CD complexes; Bortolus et al. [18] reported that for β-CD no difference in RTP could be observed between the two isomers.

Using 20 mM α-CD, the lifetimes of both enantiomers of CQ were determined by plotting the phosphorescence intensity (measured as the integral of the total phosphorescence emission spectrum) against the delay time and fitting the data with a mono-exponential function. Fig. 4 shows the decay curves (upper part) and the residual analysis (lower part) for each of the CQ enantiomers. The lifetimes thus obtained were 352 ± 16 μs for (+)-CQ, four times longer than that of (−)-CQ: 89 ± 6 μs. These values are much higher than the triplet lifetimes of α-CD–CQ complexes after laser excitation at 460 nm reported by Bortolus et al. [18] who fitted the data with a biexponential function and obtained τ_1 = 4 ± 1 μs and τ_2 = 55 ± 5 μs for (+)-CQ and τ_1 = 5 ± 1 μs and τ_2 = 45 ± 5 μs for (−)-CQ. However, it is not easy to compare the efficiencies of the solvent deoxygenation systems. In fact, our data are in line with the results reported by Romani et al. [24] for racemic mixtures of CQ in various solvents providing lifetimes ranging from 190 μs in methylcyclohexane to 430 μs in acetonitrile. Furthermore, it should be taken into account that in Bortolus' experiments a relatively low CD concentration was used ([α-CD]/[CQ] = 1.8, as compared to 10 in the present work).

The phosphorescence and fluorescence excitation and emission spectra of different mixtures of CQ enantiomers are displayed in Fig. 5. As expected, the fluorescence spectra, which were much weaker than the phosphorescence spectra (compare the vertical axes in Fig. 5a and b), showed practically no differences in intensity between the two enantiomers. This result suggests that dissociation of the 2:1 complexes takes place at the micro- to millisecond time scale. Such a dissociation rate would strongly affect the phosphorescence yield and lifetime, whereas it is too slow to affect the fluorescence parameters significantly. According to the literature [18], upon dissociation of the 2:1 complex to the 1:1 complex, the triplet state of CQ will no longer be sufficiently protected from the aqueous environment, which implies that

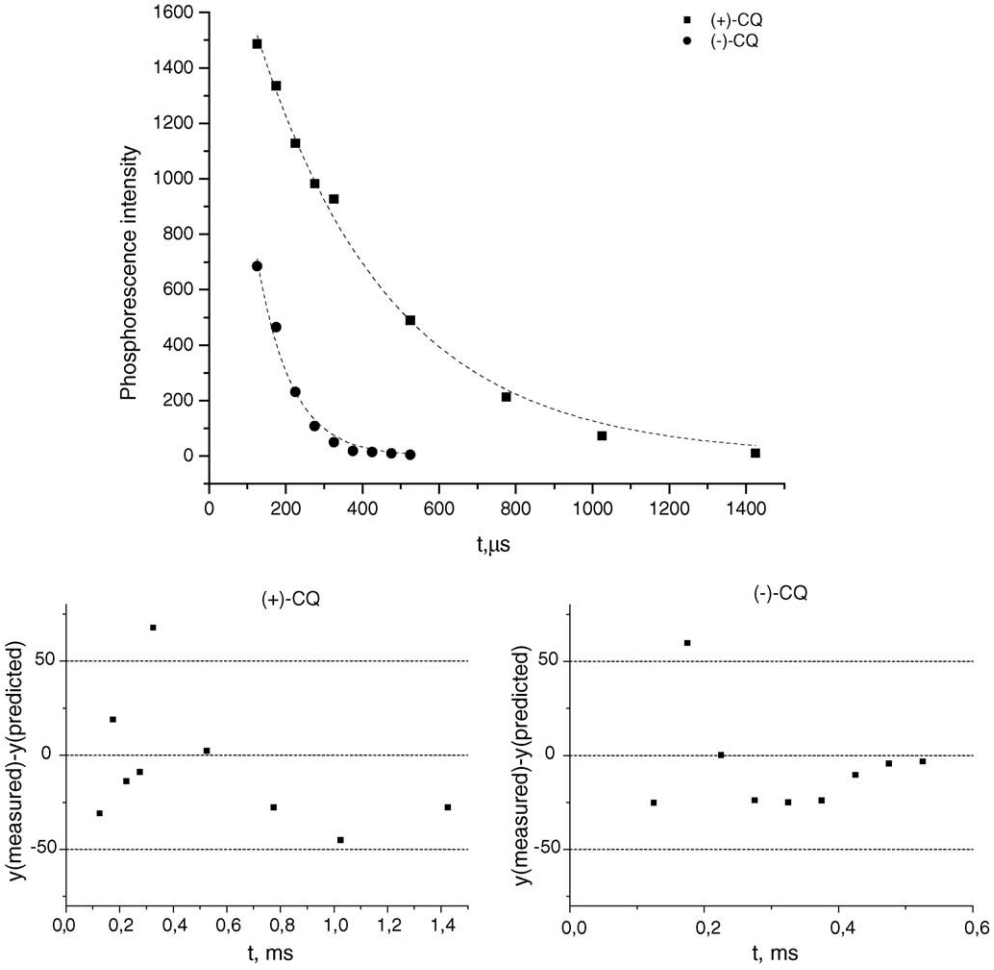

Fig. 4. Exponential decay curves and residual analysis for the phosphorescence lifetimes of CQ enantiomers.

such a partial dissociation leads to quenching of the phosphorescence. In other words, the phosphorescence lifetime will be reduced from τ_0, the lifetime in absence of dissociation (equal for both enantiomers) to τ, where $\tau = 1/\tau_0^{-1} + k_D$ and k_D is the rate constant of dissociation. Unfortunately, the dissociation rate constants for the two enantiomers, $k_D(+)$ and $k_D(-)$, can only be determined if τ_0 is known, which is not the case. Nonetheless, they can be estimated by considering two extreme cases. First, assume that τ_0 is as high as 430 μs, the highest triplet lifetime of CQ reported in organic solvents [24]. In that case, $k_D(-)$ is $8.9 \times 10^3\ s^{-1}$ and $k_D(+)$ is $5.2 \times 10^2\ s^{-1}$. In the other extreme, we can assume that for the (+)-enantiomer dissociation plays no role at all so that τ_0 is 352 μs, the value determined in this study. In that case, $k_D(+)$ is zero, while $k_D(-)$ is $8.4 \times 10^3\ s^{-1}$. We can conclude that $k_D(-)$ is in the range $(8.4–8.9) \times 10^3\ s^{-1}$ while for the (+)-enantiomer, $k_D(+) \leq 5.2 \times 10^2\ s^{-1}$. Thus, with the RTP method developed in this work one can directly detect differences in dissociation rates between (+)-CQ and (−)-CQ, which was not possible with fluorescence due to the small differences between the fluorescence spectra of the CQ enantiomers [18].

Because of the lifetime difference observed for (+)-CQ and (−)-CQ, the RTP technique could be used to determine the stereochemistry of an unknown CQ sample or the enantiomeric composition of a CQ mixture. Unfortunately, the RTP signals obtained were relatively weak and noisy, especially when long delay times and narrow gating times were used, so that a high precision could not be achieved. The most straightforward procedure was to use a delay time setting of 500 μs, measuring the (+)-CQ enantiomer exclusively. In a mixture of 2 mM CQ total concentration, the detection limit of (+)-CQ (S/N = 3) was found to be 0.25 mM, which implies that at this CQ concentration level the enantiomeric purity cannot be established more precisely than 13%. Of course at shorter delay times somewhat better precision is achievable, but obviously the precisions of 1% or even better than that are often required in analytical enantiomeric purity determinations will not be reached. Nonetheless, the RTP technique would be sufficiently selective to monitor changes in the enantiomeric ratio during biodegradation processes by microorganisms or to study the racemization of one of the enantiomers without involving a separation technique.

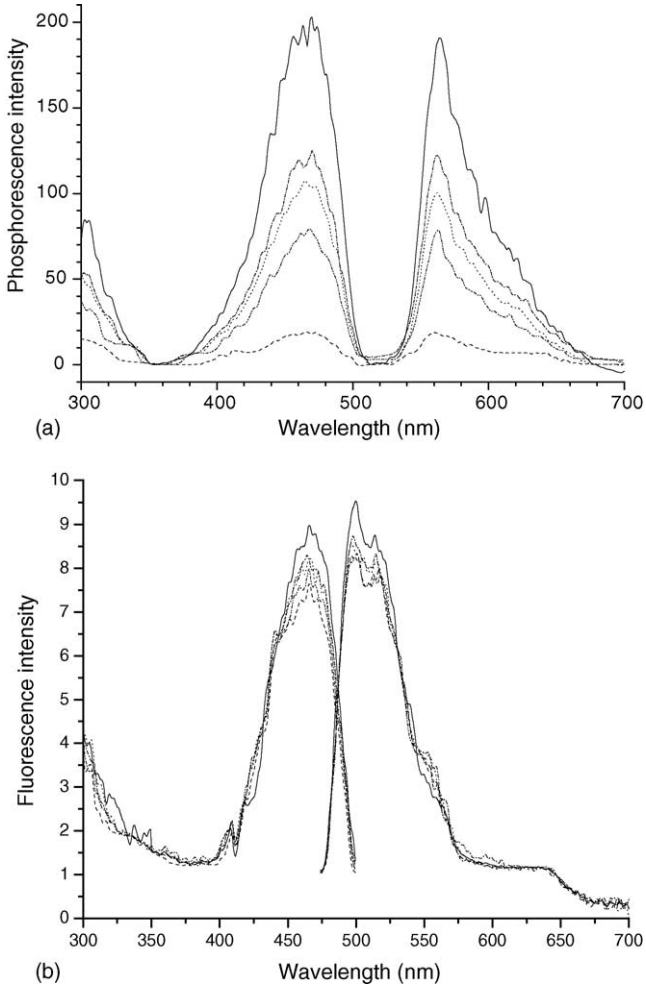

Fig. 5. Phosphorescence (a) and fluorescence (b) excitation and emission spectra of mixtures of CQ enantiomers: (—), (+)-CQ; (- - -), (−)-CQ; (· · ·), (+)-CQ:(−)-RCQ = 1:1; (- · - · -), (+)-CQ:(−)-CQ = 2:1; (- · · -), (+)-CQ:(−)-CQ = 1:2. Total CQ concentration was 2.0 mM in water with 20 mM α-CD. Phosphorescence settings as in Fig. 3. Fluorescence settings: λ_{exc} = 440 nm, λ_{em} = 530 nm; excitation bandwidth: 10 nm; emission bandwidth: 5 nm.

4. Conclusions

For the optically active analyte camphorquinone, phosphorescence of its inclusion complex with α-CD in deoxygenated aqueous solutions is remarkably enantioselective, apparently because the dissociation rate of the 2:1 complex corresponds with the phosphorescence emission time scale. In the α-CD complex, the lifetime for (+)-CQ is four times higher than for (−)-CQ. It enables easy spectroscopic discrimination between the optical isomers, based on simple instrumentation (pulsed light source and time-resolved detection), without the need to involve separation techniques.

As far as the applicability range is concerned, presumably the 2:1 complex configuration plays a crucial role in the RTP lifetime differences between the enantiomers. For the other CD complexes, which have 1:1 structures, no such differences were found. It should be expected that for other optically active analytes exhibiting RTP [26] in 2:1 CD complexes similar results may be obtained.

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