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A New Simple Procedure for Discriminating between Deracemization and an Induced CD Effect in Chiral Recognition Experiments on Atropoisomers

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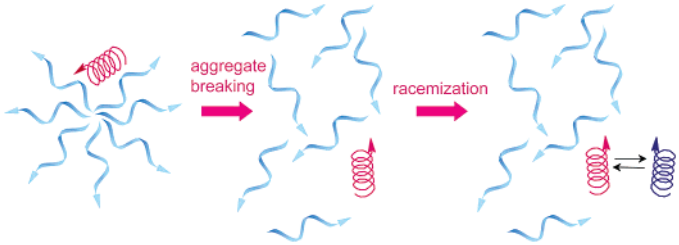
ABSTRACT



A CD band in chiral recognition experiments on racemic stereolabile compounds can be ascribed either to deracemization or to a solely induced CD effect. A procedure is presented that allows one to discriminate positively between the two phenomena. The procedure, based on CD spectroscopy, was used in experiments on racemic biphenylic derivatives in aggregates formed by enantiopure surfactants. In addition to demonstrating a deracemization event, the procedure allowed us to measure the enantiomeric excess.

Deracemization of atropoisomeric compounds in the presence of chiral aggregates such as micelles or liposomes may be an efficient model for investigating chiral recognition in biomembranes. In fact, recent papers report about the observation by CD of atropoisomer deracemization induced by chiral aggregates.[1] However, in CD experiments relative to the racemic mixture of an atropoisomeric compound in a chiral environment, the observation of a dichroic band can be due to deracemization of the racemic mixture, to an induced effect (ICD),[2] or to the superimposition of both phenomena. In some reported cases, the nature of the observed CD bands can be clarified by means of appropriate analytical tools[3] such as NMR or HPLC, but in most cases it is assessed on the basis of theoretical considerations[4] or indirect evidence.[1]

Here we describe a procedure that allows one to assess whether, in chiral recognition experiments on stereolabile compounds, a CD band is due to deracemization or solely

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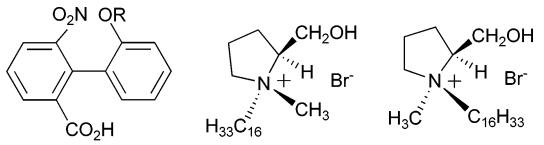
to an induced CD effect. Moreover, we report the application of this procedure in deracemization experiments carried out on racemic 2′-alkyloxy-2-carboxy-6-nitrobiphenyls, **1**, in aggregates formed by two diasteromeric cationic surfactants, *N*-hexadecyl-*N*-methyl-L-prolinolinium bromide **2**.



1a R=CH_3
1b R=$C_{12}H_{25}$ **2a** **2b**

Both deracemization and ICD are due to tight interactions between the chromophore and the chiral agent or aggregate; breaking of such interactions by addition of a solvent suitable to disrupt the aggregates will not leave any trace of an induced effect but will leave an enantiomeric excess (ee) as a trace of deracemization. This ee will be detectable by CD if the racemization process is not too fast. Since the half-life for the racemization process of compounds **1a**[5] and **1b**[6] at 298 K is 25 and 150 min, respectively, in our investigations it will be possible to observe a CD band soon after aggregate breaking if deracemization occurred. The procedure we used consists of (a) freezing the samples in liquid nitrogen in order to take a snapshot of the possible enantiomeric excess; (b) adding an excess of methanol[7] and 5% trifluoracetic acid[8] at low temperature (258 K) in order to disrupt the aggregates; (c) running a CD spectrum of the obtained solution as soon as the solution appears to be homogeneous. The presence of a band in the CD spectrum of the solution obtained after aggregate breaking indicates that the aggregates deracemized the racemic biphenylic derivative, i.e., inside the aggregates the equilibrium between the enantiomers was shifted toward one of the two. In such a case, the enantiomeric excess, gained inside the aggregate, can be easily obtained by the CD spectrum recorded immediately after aggregate breaking, provided that the chiroptical and spectroscopic properties (molar ellipticity and extinction coefficient) of the solute in the mixture obtained by aggregate breaking are known.

This procedure is general and can be applied to many supramolecular systems (conditions of step b may be changed according to the system under investigation), provided the half-life for the racemization of the dichroic atropoisomer is such that it allows the registration of a CD spectrum soon after aggregate breaking. As a matter of fact, in the presence of a reasonable extent of deracemization, it can also be used to measure the rotational barrier of the investigated atropoisomer by following the decay of the CD band over time, due to the racemization process, after disruption of the supramolecular system.

The CD bands observed under aggregating conditions[9] on racemic 8.0 mM 2-carboxy-2′-methoxy-6-nitrobiphenyl, **1a**, in aqueous solution 40.0 mM in either cationic surfactant **2a** or **2b** are reported in Figure 1. It can be observed that

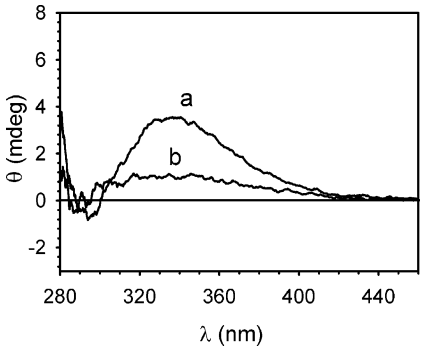


Figure 1. CD spectra at 309 K (cell path length 0.1 cm) of a 200 mM NaCl aqueous solution of 8.0 mM **1a** in the presence of: (a) 40.0 mM surfactant **2a**; (b) 40.0 mM surfactant **2b**.

the CD band of biphenylic derivative **1a** is more intense in the presence of the aggregates formed by **2a**. This behavior is analogous to that previously observed in the case of compound **1b**.[1b] However, the intensity of the CD bands relative to **1a** is approximately one-third with respect to those relative to **1b**.

We carried out other CD experiments on both atropoisomers, following the variation of the ellipticity of the biphenylic derivative (4.0 mM) as a function of surfactant **2a** concentration. Results of these experiments are remarkably different for the two solutes (Figure 2). Increasing the

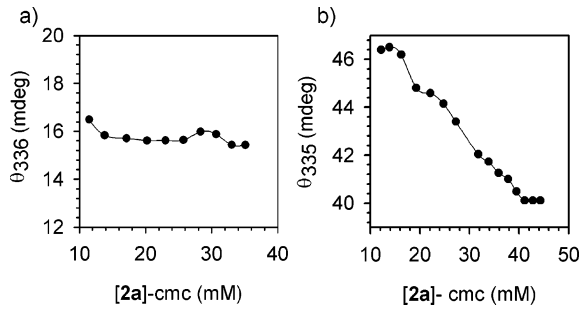


Figure 2. CD band intensity (cell path length 0.5 cm) as a function of aggregated surfactant **2a** concentration (analytical concentration subtracted from critical micellar concentration, cmc) of an aqueous solution of: (a) 4.0 mM **1a**; (b) 4.0 mM **1b**.

surfactant concentration does not change the observed ellipticity of compound **1a** (Figure 2a) but decreases that of compound **1b** (Figure 2b).

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(7) It was easily observable by ^{1}H NMR experiments that the signal line width narrowed abruptly following the addition of 5 vol of methanol, indicating an immediate breaking of the aggregate. The relative NMR spectra are available in Supporting Information.

(8) Sakamoto, K.; Masahiro, H. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 339. Trifluorocetic acid in 5% of the solution volume was added also to ensure protonation of the carboxylic group.

The procedure of aggregate breaking allowed us to reveal unequivocally that deracemization occurred under aggregating conditions. In fact, in all the performed experiments, after disruption of aggregates, we could observe a CD band whose intensity decreased to zero with the racemization rate expected for compounds **1a** and **1b**, respectively.[5,6] The extent of deracemization was easily measured because the chiroptical properties of the two compounds were determined in MeOH/H_2O/5% TFA.[10] Therefore, from the intensity of CD bands in the spectra obtained after disruption of aggregates, we obtained the concentration of the exceeding enantiomer, whereas from the UV spectrum, we obtained the total concentration of biphenylic derivative and, as a consequence, the ee.

The procedure of aggregate breaking on samples relative to the experiments described in Figure 1 (compound **1a**) gives evidence that the ee is 2% in the presence of aggregates formed by **2a** and below 1% in the presence of aggregates formed by **2b**. The same procedure applied on analogous samples containing **1b** confirmed a deracemization phenomenon, previously assessed only on the basis of indirect evidence,[1b] with 6 and 2% ee in the presence of aggregates formed by **2a** and **2b**, respectively.

Regarding the experiments performed at different **2a** concentrations (Figures 2a and 2b), the procedure of aggregate breaking revealed that for both compounds **1a** and **1b**, the ee does not change as a function of surfactant concentration. The measured ee relative to **1a** was 2%, and those relative to **1b** were 6%. The constant ee found in the experiments relative to **1b** demonstrates that the pattern observed in aggregating conditions (Figure 2b) is due to a change in the molar ellipticity as a consequence of the different organization of the aggregates. This result is confirmed by the UV spectra of the same samples that show a variation of the biphenyl absorbance as a function of surfactant concentration.

As demonstrated, the above-described procedure reveals deracemization in chiral recognition investigations and, for substrates with high molar ellipticity, also allows detection of very low extents of deracemization that could not be revealed by means of other techniques such as NMR or HPLC.

The measured ee, though modest, gives evidence that surfactant **2a** has a greater ability to deracemize the investigated racemic mixtures than surfactant **2b**. The extent of deracemization depends on the solute as well; in fact, the presence of the hydrophobic chain in the 2′-position (compound **1b**) of the biphenylic moiety is found to be an important feature because it locks the solute onto the aggregate with a more favorable topology than derivative **1a**.

The nature of the solute and its mode of binding also influence the organization and morphology of the aggregates, as evidenced by dynamic laser light scattering (DLS) experiments (Table 1). It can be observed that the presence of the solute induces the formation of larger and probably more organized aggregates. However, this effect is remarkably stronger in the presence of the biphenylic derivative **1b** (entries 4 and 6), the relative size of the aggregates suggesting the formation of vesicles. In particular, in the case of aggregates formed by surfactant **2a** in the presence of biphenylic derivative **1b**, DLS experiments seem to indicate the presence of two size distributions centered at a hydrodynamic radius of 25 and 60 nm, respectively. This peculiar result was confirmed by the transmission electron microscopy (TEM) experiment reported in Figure 3. The TEM picture clearly shows in white the shape of the vesicles because the contrast agent (2% w/v solution of phosphotungstate acid) localizes outside of the structures to be imaged; it is evident that there are two major populations, with a diameter of 25 and 60 nm, respectively.

Table 1. Hydrodynamic Radius of Aggregates Formed by 40.0 mM Surfactant **2a** or **2b** in the Presence of and in the Absence of 8.0 mM Biphenyls **1a** or **1b**, in 200 mM Aqueous NaCl, Obtained at $T = 309$ K by DLS

entry	surfactant (40 mM)	solute (8.0 mM)	r_h, nm
1	**2a**		3.1
2	**2b**		2.9
3	**2a**	**1a**	6.7
4	**2a**	**1b**	25/60
5	**2b**	**1a**	3.5
6	**2b**	**1b**	25

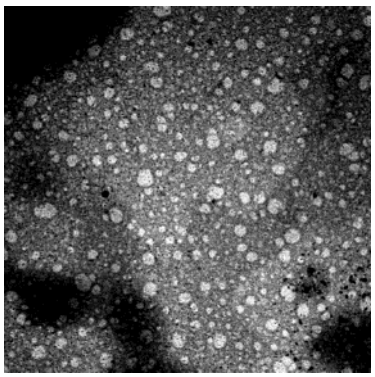

Figure 3. TEM picture of a 200 mM aqueous NaCl sample, 8.0 mM in **1b** and 40.0 mM in **2a** (frame 2329 nm).

The association of the solute modulates, at the same time, the morphology of the aggregates and their recognition

(9) For the preparation of compounds **2** and **1b** as well as for experimental details of the preparation of the samples, see ref 1b. For the preparation of compound **1a**, see ref 5.

(10) After resolution of the racemic mixture of **1a** and **1b** by HPLC on a chiral stationary phase as previously described for **1a** (see ref 5), the chiroptical properties of the two compounds in MeOH/H_2O/5% TFA were determined. The molar ellipticity at 330 nm is 7987 ± 468 and 7917 ± 477 deg $dmol^{-1}$ cm^2 for compounds **1a** and **1b**, respectively. The molar extinction coefficient at 274 nm is 3740 ± 32 for **1a** and 4336 ± 42 M^{-1} cm^{-1} for **1b**. The complete CD spectra of **1a** and **1b** are available in Supporting Information.

capability, so that the extent of chiral recognition is apparently independent of the amount of surfactant.

The collected results demonstrate the complexity of the systems investigated. In these systems, recognition cannot be ascribed to simple noncovalent interactions between the monomers, behaving independently one from the other, and the solute but instead depends on the assembly in its whole. Moreover, chiral recognition may depend not only on specific interactions of the chiral solute with portions of the monomers close to the stereocenters but also on interactions with regions of the aggregate in which a chiral environment is induced by a complex texture of noncovalent interactions, governed by the contribution of the aggregated chiral monomers. It is consequently important to also identify modest recognition phenomena in order to shed some light into the complexity of the interactions governing self-assemblies of amphiphilic molecules.

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Supporting Information Available: Experimental data and CD and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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