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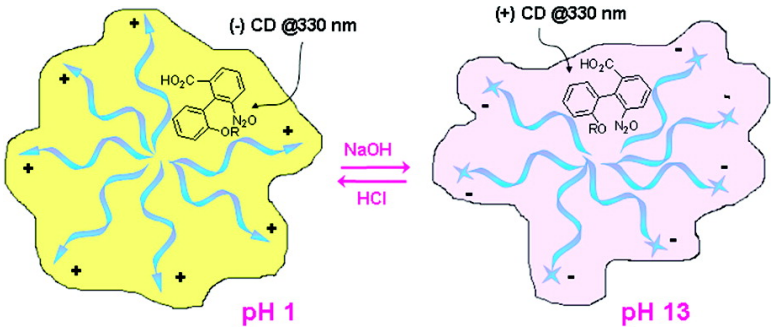

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pH Variation as the Switch for Chiral Recognition in a Biomembrane Model

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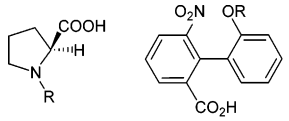
Life is intimately linked to homochirality, and chiral recognition governs most biochemical processes. At the molecular level, chiral recognition has been widely studied and is fairly well understood, whereas the transfer of chirality to the various organizational levels of multicomponent chiral biological systems is mostly still unknown, though pivotal investigations have been carried out on different systems and models.[1] In this regard, an aspect that still needs to be elucidated is the relationship between the organization of the biological membranes and the molecular structure of the membrane components. Chirality is information codified in the molecular structure and plays a fundamental role in the membrane organization through recognition processes,[2] but it is still unclear how the stereochemical information controls the organization and functions of cell membrane, how chirality, like other functions codified in the monomers, is transferred from the molecule to the assembly, and how it runs at a higher level of organization. Because of biomembrane complexity, the investigations of these systems requires the use of models, such as micelles and liposomes, where the interactions responsible for organization are the same as those involved in a biomembrane, but where chemical composition and complexity are under control. We have been investigating chiral recognition in micellar aggregates formed by chiral surfactants[3] exploiting, as markers of chiral recognition, the deracemization of chiral biphenylic derivatives,[3a,c] the recognition of chiral dipeptides,[3d] or the induced circular dichroism of a chromophore, such as a porphyrin,[3b] and obtained, in some cases, evidences suggesting a site of expression of chirality in the assembly far from the stereogenic centers of the surfactant monomers.[3b,d]

Here, we report on the investigation of the expression of chirality in aggregates formed by *N*-dodecyl-L-proline, **1a**, a surfactant that has been largely used as a chiral selector for enantioselective separations.[4] As a probe of chirality transfer, we used the axially chiral biphenyl **2a**, whose deracemization was monitored by ^{1}H NMR, circular dichroism (CD), and enantioselective HPLC. Within this system, stereochemical information is transferred from the chiral assembly of **1a** to the biphenyl probe and manifests itself as an imbalance in the 1:1 equilibrium ratio of the interconverting enantiomers of the probe.

The extent of observed deracemization was, to the best of our knowledge, the highest ever observed in polymolecular aggregates and showed a strong dependence on pH and concentration conditions.

Deracemization experiments were carried out on aqueous samples, 50 mM in **1a**, under different pH conditions, at concentrations of **2a** corresponding to a [**1a**]/[**2a**] ratio in the range of 10−5000.



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

The NMR spectra of the considered samples feature a line width enlargement that does not allow the detection of signal splittings due to externally heterotopic nuclei; only the spectrum of the sample at a [**1a**]/[**2a**] = 45 ratio at pH 7 suggested the presence of split signals and was better investigated at 600 mHz. The spectrum reported in Figure 1 shows distinct signals for the enantiomers of **2a** corresponding to hydrogens in 6′ (7.40 ppm) and 3′ (6.87 ppm) positions. The resolution of the signals does not allow measuring of their integrals, but the deconvolution of signals due to 3′ hydrogen suggests a ∼20% enantiomeric excess (ee).

The CD spectra of all the samples show a band centered at 330 nm, whose sign and intensity depend on the pH conditions. As an example, in Figure 2, we reported the CD spectra performed under different pH conditions at [**1**]/[**2**] = 45 ratio.

The presence of a CD band in such systems could be due to deracemization of the biphenylic derivative, to an induced CD effect (ICD) or to the superimposition of both phenomena. We ascertained and measured deracemization by HPLC, using a chiral stationary phase. HPLC not only allowed us also to measure the enantiomeric excess in all the examined samples (ee's in Table 1), but it served to securely identify which is the enantiomer in excess in the deracemized mixtures. Direct injection of equilibrated acidic and basic solutions showed an excess of the first eluting enantiomer at 1 < pH < 7, while the opposite was observed at pH > 7 (the chromatograms are available as Figure 1S of Supporting Information). The HPLC results clearly indicate that CD sign reversals following extreme pH changes are not due to a conformational change of **2** but rather to a shift in the stereochemical preference of the chiral aggregate accompanying acidic/basic transitions of the medium.

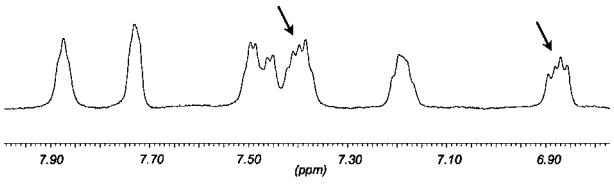


Figure 1. Region of the ^{1}H NMR spectrum relative to aromatic signals of a D_2O solution, 1.1 mM in **2a** and 50.0 mM in **1a** at pH 7 (Bruker Avance AQ600 spectrometer).

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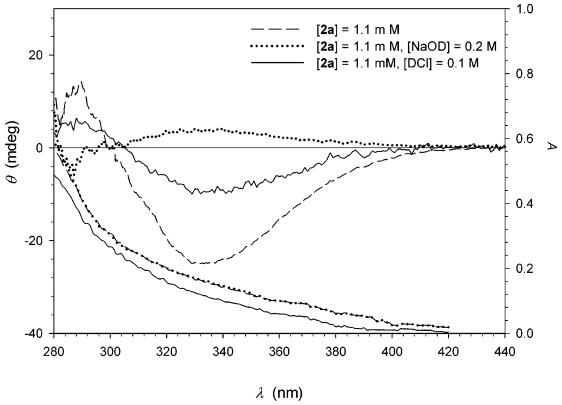

Figure 2. CD (0.5 cm path length cell) and UV−vis (0.1 cm path length cell) spectra of D_2O solutions, 50 mM in **1a** and 1.1 mM in **2a** in different pH conditions.

Table 1. Enantiomeric Excess (ee) of **2a** as a Result of Deracemization Due to the Chiral Aggregate Formed by 50 mM **1a** under Different pH Conditions (the sign in brackets refers to the sign of the CD band of the exceeding enantiomer)

ratio [**1a**]/[**2a**]	[**2a**] mM	pH 1	pH 7	pH 13
10	5.0	(−) 5%	(−) 3%	(+) <2%
45	1.1	(−) 5%	(−) 20%	(+) <2%
91	0.55	(−) 5%	(−) 20%	(+) 3%
455	0.11	(−) 5%	(−) 22%	(+) 8%
5000	0.010	(−) 5%	(−) 22%	(+) 21%

Changes in the stereochemical bias upon pH variations are reversible. A complete cycle could be followed by HPLC on a sample containing 0.010 mM **2a** starting from a basic medium (0.2 M NaOH). The sample was made neutral and acidic and was brought back to alkaline pH by controlled additions of concentrated HCl and NaOH; the relative peak areas of the equilibrated enantiomers were measured by HPLC at each pH, and the resulting values matched those obtained from samples prepared separately at different pH conditions. Parallel CD studies were carried out on a sample containing 1.1 mM **2a**, and the resulting spectra matched those reported in Figure 2.

Results of deracemization experiments reported in Table 1 indicate that the favored enantiomer depends on the pH of the bulk, demonstrating that the deracemization process is governed by the charge features of the aggregates.[5] Moreover, the extent of deracemization depends on pH conditions as well as on the [**1a**]/[**2a**] ratio. At pH = 7, in fact, a minimum [**1a**]/[**2a**] ratio of 45 is required for observing the highest extent of deracemization; under basic conditions, the necessary [**1a**]/[**2a**] ratio for observing the highest ee is 5000, whereas at pH = 1, the extent of deracemization is modest and not influenced by the [**1a**]/[**2a**] ratio. In some conditions, the obtained ee's are higher than those usually observed in these model systems.

The association of **2a** with the aggregates is very high in all pH conditions. In fact, at low pH values, in the presence of surfactant **1a**, the neutral form of **2a**, though insoluble in water, gives clear solutions.[6] On the other hand, under basic conditions, **2a** is soluble in water and micellizes above a 2.8×10^{-5} M concentration (cmc);[7] hence, in the presence of **1a**, we can reasonably assume that it will form mixed aggregates. The mode of association of **2a** with the aggregate is then controlled by the structure of the aggregate itself as well as by its own state of charge and, hence, by the equilibrium between its neutral and charged forms that may associate with different sites of binding.

The recognition process in not due to a direct interaction of the aromatic portion of the solute with the chiral center of a single headgroup because in some pH conditions a minimum [**1a**]/[**2a**] ratio is required for observing a high deracemization. Moreover, blank experiments carried out on **2a** (1 mM) and on its analogue **2b** (1 mM), under alkaline conditions (0.2 M NaOD), in 50 mM *N*-methylproline **1b**, gave, as expected, no evidence of deracemization. The aromatic region of the ^{1}H NMR spectrum shows only one set of signals in the experiment relative to **2b** and scarcely resolved signals in that relative to **2a** (see Figure 2S of Supporting Information). The decisive response came from CD spectra that did not show any band, and from HPLC chromatograms that showed racemic mixtures in both experiments. Therefore, recognition has to be ascribed to a complex texture of interactions with a whole region of the aggregate that we can define as "chiral pocket", whose structure is due to a cooperative effect of the chiral headgroups and of assembling.

The collected evidences support the hypothesis that, in this model system, the chiral function is expressed as a chiral cavity that results from the contribution of the whole aggregate and that reversibly changes its shape and probably its location as a function of pH.

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Supporting Information Available: Details on instrumentation and procedures. HPLC chromatograms. ^{1}H NMR of blank experiments. UV−vis spectra of surfactant **1a** and CD and UV−vis spectra of biphenyl derivatives **2a** and **2b**. This material is available free of charge via Internet at http://pubs.acs.org.

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