Circular dichroism spectra were recorded for micellar aggregates of N-stearoyl (L or D) serine in H2O or D2O. Micelle formation kinetics differed markedly in H2O, but in D2O the enantiomers showed similar spectral characteristics. The results confirm previous observations (1) that described differences in the thermodynamic properties of enantiomers, in contrary to the reigning dogma. The comparison of spectral properties indicates that this phenomenon depends on the interactions with H2O and is not due to trivial contamination.
Mirror symmetry breaking in micelles of N-stearoyl serine enantiomers

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*Dedicated to the memory of Proff. Meir Shinitzky - his path I follow, on his shoulders I stand.

Non-identity of enantiomers, in contrary to the existing dogma, is proved by this work

Abstract: Circular dichroism spectra were recorded for micellar aggregates of N-stearoyl (L or D) serine in H$_2$O or D$_2$O. Micelle formation kinetics differed markedly in H$_2$O, but in D$_2$O the enantiomers showed similar spectral characteristics. The results confirm previous observations (1) that described differences in the thermodynamic properties of enantiomers, in contrary to the reigning dogma. The comparison of spectral properties indicates that this phenomenon depends on the interactions with H$_2$O and is not due to trivial contamination.

Main

The origin of biological homochirality incorporating L-amino acids and D-sugars remains an unanswered question that has been discussed in numerous monographs and reviews (2-12). One approach is that whatever the cause, the presumed identity of the physical properties of enantiomers make spontaneous symmetry breaking a matter of chance, so that life could equally well have incorporated D-amino acids and L-sugars (3).
An alternative view posits that symmetry breaking is deterministic. For instance, it is now established that left–right symmetry is broken at the level of interactions between subatomic particles and atoms, and it is possible that this fundamental dissymmetry can be transferred to the molecular scale (2). The spectral characteristics and a crucial comparison with D$_2$O strongly support the existence of significant differences between enantiomers in their interactions with H$_2$O, but not with D$_2$O.

**Methods**

*Preparation of stearoyl serine solutions*

Stearic acid, N-hydroxysuccinimide, ethyl acetate, dicyclohexylcarbodiimide, ethanol, tetrahydrofuran, L and D serine, sodium bicarbonate, hydrochloric acid and acetone were purchased from Sigma Aldrich. The chiral surfactants, D- and L-stearoyl serine, (D-NSS and L-NSS) were prepared by coupling stearic acid N-hydroxy succinimide ester with D and L serine, as described by Lapidot et al (11). The structures and compositions of the D-NSS and L-NSS were confirmed by $^1$H-NMR, mass spectroscopy, infrared spectra and optical rotation. Mass spectroscopy showed that the purity of the D and L stearoyl serine compounds exceeded 98.5%.

Initial solutions of L-NSS and D-NSS were prepared in 1:1 ethanol:water at a concentration of 0.1 M. Their concentrations, calibrated by absorption spectra, were found to be identical within the limits of instrument resolution. An aliquot of the respective initial solution was mixed 1:1000 (18 µL into 1800 µL) in H$_2$O and D$_2$O (0.01 M KOH solutions) to form a working solution $10^{-4}$ M of L-NSS and D-NSS.
Circular dichroism measurements

CD measurements using a 1 mm quartz cuvette were performed with a Chirascan spectrophotometer (Applied Photophysics) from 280 to 196 nm in 1 nm steps, 3 seconds per point, and 4 minutes between successive runs. Measurements began two minutes after the working solutions were prepared, and the baseline of the solvent was subtracted.

Controls for impurities

To determine whether impurities present in the water were somehow affecting the measurements, the double distilled water (DDW) was evaporated until 1/20 of the initial volume remained, so that any impurities would be concentrated 20-fold. The residual water was then used to prepare and monitor micelle formation. The results were identical to those with DDW, weighing against the possibility that unknown contaminants in the water were causing the observed differences.

Results

Figure 1 shows the CD spectra of the two enantiomers in 1:1 methanol/water KOH 0.01M, which are conditions that suppress micelle formation. The spectra are identical, confirming that the differences observed in CD spectra when micelles are able to form are not a consequence of impurities or variations in the way the solutions are prepared.
Figure 1. Circular dichroism of D-NSS (D-stearoyl serine, upper curves) and L-NSS (L-stearoyl serine, lower curves) in 0.01M KOH in H₂O, mixed 1:1 with methanol. These conditions inhibit micelle formation and the spectra are identical.

When conditions conducive to micelle formation are employed, there is a clear difference in the rates of micelle formation between L-NSS and D-NSS in H₂O, as depicted in Figure 2. The spectra of L-NSS micelles change only slightly over time, as shown by the repetitions, to about 32 millidegrees. In contrast, the spectra of D-NSS micelles in H₂O grow markedly over time and at a crucial size change in shape. The magnitude of D-NSS is also higher, at approximately -62 millidegrees.
Figure 2. Circular dichroism of L-NSS (L-stearoyl serine, upper curves) and D-NSS (D-stearoyl serine, lower curves) in 0.01M KOH in H₂O. These conditions promote micelle formation. Each spectrum is separated from the next by four minutes.

In D₂O the spectra of D-NSS resembles its spectra in H₂O spectra as illustrated in Figure 3. However, the spectra of L-NSS are markedly different from those in H₂O and now more closely resemble the D-NSS spectra.
**Figure 3.** Circular dichroism of L-NSS (L-stearoyl serine, upper curves) and D-NSS (D-stearoyl serine, lower curves) in 0.01M KOH in D$_2$O. These conditions promote micelle formation. Each spectrum is separated from the next by four minutes.

In order to illustrate the differences in kinetic behavior of L-NSS and D-NSS, time points were plotted for the spectral shifts of the two enantiomers in H$_2$O and D$_2$O as depicted in Figure 4. The L-NSS reaches its equilibrium value within two minutes in H$_2$O, but more slowly in D$_2$O, whereas D-NSS' kinetic behavior is similar in H$_2$O and D$_2$O and requires significantly more time to reach equilibrium.
Figure 4. Circular dichroism of L-NSS (L-stearoyl serine) in H₂O (223 nm), L-NSS (L-stearoyl serine) in D₂O (206 nm), D-NSS (D-stearoyl serine) in H₂O (206 nm) and D-NSS (D-stearoyl serine) in D₂O (206 nm), versus time (represented by repeating the measurement every four minutes).

The identity in structure as seen in the CD spectra between D-NSS micelles in DDW and in D₂O can be visualized using electron microscopy, as clearly shown in Figure 5. Furthermore, the shift in structure of L-NSS micelles in D₂O, compared to the structure in DDW, to identity with the D-NSS structure, is illustrated. D-NSS micelles begin forming a structure resembling the L-NSS micelles in DDW and a shift in structure occurs during formation in agreeance with the CD spectra. The difference in structure between L-NSS micelles in DDW and D-NSS micelles in DDW is distinct.
Figure 5. Electron micrographs of L-NSS and D-NSS micelles in DDW. The micelles were prepared by the same technique described in Methods. Briefly, an aliquot from the respective solutions were sampled 8 and 30 minutes after beginning of the micellar formation. Specimens were prepared for electron microscopy using the standard staining method. A. L-NSS micelles after 30 minutes; B. D-NSS micelles formation after 8 minutes (at the right upper side of the picture remnants of structures akin to the L-NSS, can be seen); C. L-NSS micelles in D$_2$O after 30 minutes. The shift from the structure in DDW to a structure identical to D-NSS micelles structure (in DDW and D$_2$O) is shown; D. D-NSS micelles in D$_2$O after 30 minutes; E. D-NSS micelles in D$_2$O after 30 minutes.
Discussion

Studies from the Shinitzky group have suggested that hydration shells around chiral amino acids in water could enhance chiral discrimination (1, 13). In the case of polyaminoacids and peptides dissolved in water, differences between poly-L and poly-D peptides were observed in helix-coil transition (1) as well as in thermal stability and supramolecular structures (14). When tested in D₂O under identical conditions these differences were markedly reduced, indicating a specific discrimination between chiral configurations by H₂O molecules. It has been proposed (1, 13) that the spin isomers of water, i.e. ortho-H₂O and para-H₂O, present in a 3:1 ratio could account, at least partially, for this discriminatory power. In D₂O the spin isomers are much less pronounced, which drastically attenuate the putative chiral preference.

Several publications from other investigators support this notion. In 2008, Kodona et al reported that the α-helical motif in poly-D-glutamic acid is stable thermally, compared to that of poly-L-glutamic acid. This difference was ascribed to an amplified interaction of the L-species with the more abundant ortho-water (14). Guo et al investigated the dilution enthalpies of enantiomers of six beta-amino alcohols in dimethylsulfoxide (DMSO) + H₂O mixtures using an isothermal titration calorimeter, and calculated the corresponding homochiral enthalpic pairwise interaction coefficients (hXX) of the six amino alcohols. It was found that throughout the composition range of mixed solvents, values of hXX for the S-enantiomer are virtually all greater than those of R-enantiomer for each amino alcohol (15). Fujiki et al investigated several ambidextrous artificial helical Si–Si bonded polysilanes in solution as a function of temperature. Their
preliminary results indicated subtle differences in chiroptical and achiral $^{29}$Si-NMR and viscometric data for a pair of helical polysilanes (16).

*Can parity-violating energy difference (PVED) account for these variances?*

Theoretical studies (2, 4) have established that an image and its mirror molecule are not true enantiomers due to a parity-violating energy shift (PVES) of the electronic binding energy with a positive value, $+\text{EPV}$, for the image molecule and a PVES with a negative value, $-\text{EPV}$, for the mirror image molecule (or vice versa); PVES makes the pair become a diastereomer. The parity-violating energy difference (PVED) between image and mirror image molecules is given as $\text{PVED} = \text{EPV} - (-\text{EPV}) = 2\text{EPV}$.

Other studies have proposed that PVES might be detectable as slight differences in electronic transition energies (2, 17). However, because of the extremely small PVED of $\sim 10^{-19}$ eV, it is generally believed that parity violation cannot induce observable macroscopic thermodynamic differences between enantiomers. To overcome the vanishingly small PVED, several amplification models have been proposed to explain the emergence of biological homochirality (2).

*Stearoyl serine enantiomer properties are different in H$_2$O and D$_2$O*

Micelle formation of L and D-stearoyl serine and the differences between the enantiomers were discovered by Shinitzky et al (18). N-stearoyl serine micelles presumably originate from a repetitive arrangement of the amide planes on the micellar surface, forming a “quasi-peptide” bonded by hydrogen bonds, replacing peptide bonds.
The formation of the micelles by hydrogen bonds instead of strong peptide bonds makes it a very sensitive system that amplifies the influence of solvents.

The spectral kinetics of micelle formation reported here provide further support for the notion that enantiomers have non-identical thermodynamic macroscopic properties, perhaps due to parity violation amplification by the differential interaction of the enantiomers with H₂O. The observation that the differences are considerably reduced in D₂O supports this hypothesis. This putative chiral preference in H₂O may encapsulate the basic molecular mechanism of parity violation amplification which is capable of transferring PVED to the molecular level. Differential hydration of enantiomers may also have played a crucial role in the deterministic selection of L-amino acids by early forms of life.

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