Optically Active Oligomer Units in Aggregates of a Highly Unsaturated, Optically Inactive Carotenoid Phospholipid

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Abstract: Enantiomers of glycerophospholipids show low or no optical activity. Accordingly, optical activity was not observed with the *R* enantiomer of a highly unsaturated carotenoyl lysophospholipid in solution. In spite of this, strong Cotton effects are detected in water. The amphiphilic carotenoid–phospholipid monomers associate to form aggregates, whose optical activity is attributed to oligomeric entities. These small helical assemblies cannot exist independently. Yet, the calculated octamer represents the simplest repeating primary unit that sufficiently expresses the absorption properties and supramolecular optical activity.

Keywords: carotenoids · helical structures · molecular modeling · phospholipids

Introduction

The chiroptical properties of glycerolipids have been rarely investigated, while the two other groups of vital biomolecules, carbohydrates and proteins with their well-established exclusive building blocks of D-sugars and L-amino acids, have received a lot of attention. Despite the fact that the biosynthesis of lipids also results in only one specific enantiomer, a chiral, functional discrimination of enantiomeric lipids has rarely been found. Membranes consist of pure phospholipid enantiomers, but with respect to penetration and other physical properties, phosphocholines behave as achiral molecules.[1–3] It was only recently that a decisive enantiomeric interaction between odorants and lipids was detected in the olfactoric system.[4] Another reason for neglecting chiroptical investigations of glycerolipids is their invisibility. (Phospho)lipids show notoriously low or no specific rotations, likewise, electronic optical activity (EOA) is weak or absent due to the lack of chromophores.[5]

The few natural chromophoric lipids contain ajenoic acids C12:5, C14:5 in triglycerides[6] and parinaric acid C18:4 in phophatidylcholines.[7] The circular dichroism (CD), or fluorescence-detected CD (FDCD), of these lipids has not yet been measured, nor have chiral lipids been prepared from synthetic conjugated tetraenic fatty acids.[8] The acyl chains in these unsaturated lipids are only partly accessible by EOA spectroscopy. In various attempts to introduce chromophores, phospholipids with stilbene, biphenyl, terphenyl, and azobenzene fatty acids have been synthesized.[9–11] Likewise, a glycerophosphatidylcholine enantiomer with styrylthiophene acyl groups was synthesized, which was devoid of optical activity.[12] Undoubtedly, the mentioned lipids contain rather xenobiotic acyl groups and are, therefore, different from lipids of naturally occurring fatty acids.

For historical reasons optical activity is restricted to wavelengths from 200–800 nm. Although the accessible chiroptical spectral range has successively been extended to both lower and higher wavelengths, enantiomers continue to be defined as "optically inactive" when no signal in the classical wavelength scale is detected. Thus, the highly unsaturated carotenoylphospholipid (*R*)-**5** (in MeOH), which does not exhibit EOA in the usual accessible range of the dichrograph, (see Figure 1a, c), is considered optically inactive.

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However, (*R*)-**5** shows optical activity in water (Figure 1a). The chiroptical properties of (*R*)-**5** result from the self-assembly of optically inactive monomers to give supramolecular structures.[13,14] By calculating the absorption and CD spectra of a manifold of oligomeric structures, we found that a helical oligomer composed of eight monomers may serve as a basic unit that explains satisfactorily the spectroscopic properties of (*R*)-**5** aggregates.

We report here on the synthesis of lysophospholipid (*R*)-**5**, on its chiroptical properties, and the calculation of a basic aggregation unit.

Results and Discussion

Lysocarotenoylphosphatidyl choline ((*R*)-**5**) was synthesized in low yield by reacting the natural (+)-(2*R*)-glycero-3-phosphocholine (3-*sn*-GPC) ((*R*)-**1**) with the rigid and fully chromophoric carotenoic acid (C30-acid)[15,16] **2** in the presence of the imidazole derivative **3** and 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU; **4**)[17] (Scheme 1). Dissolved in methanol, the carotenoid derivative (*R*)-**5** did not show EOA (Figure 1a, c).[18] A chiral perturbation of the polyene chain is not expected to occur in (*R*)-**5** and, in addition, Cotton effects in the absorption region of the polyene chain above 400 nm are difficult to detect.[19,20] In some colorless glycerides, weak Cotton effects were observed around 220 nm (n–π* transitions of the ester group),[21–24] sometimes with opposite signs for the same enantiomer.[25] The measurement of the weak specific rotation of lysophospholipids requires high compound concentrations (3–5 %).[26] Such deep orange colored solutions of (*R*)-**5** are not transparent and, therefore, hardly detectable in the polarimeter.[27]

Optically inactive enantiomers[28,29] have been, incorrectly, called "cryptochiral", because "chirality" frequently, but inadmissibly, is considered to be equivalent with electronic optical activity.[30–34] The more appropriate and descriptive term "cryptoactive" for glyceride enantiomers without detectable electronic optical activity has been largely ignored.[5] Nevertheless, we will rank the carotenoylglycerophospholipid (*R*)-**5** to the class of cryptoactive compounds or to compounds with *accidental optical inactivity*.[35]

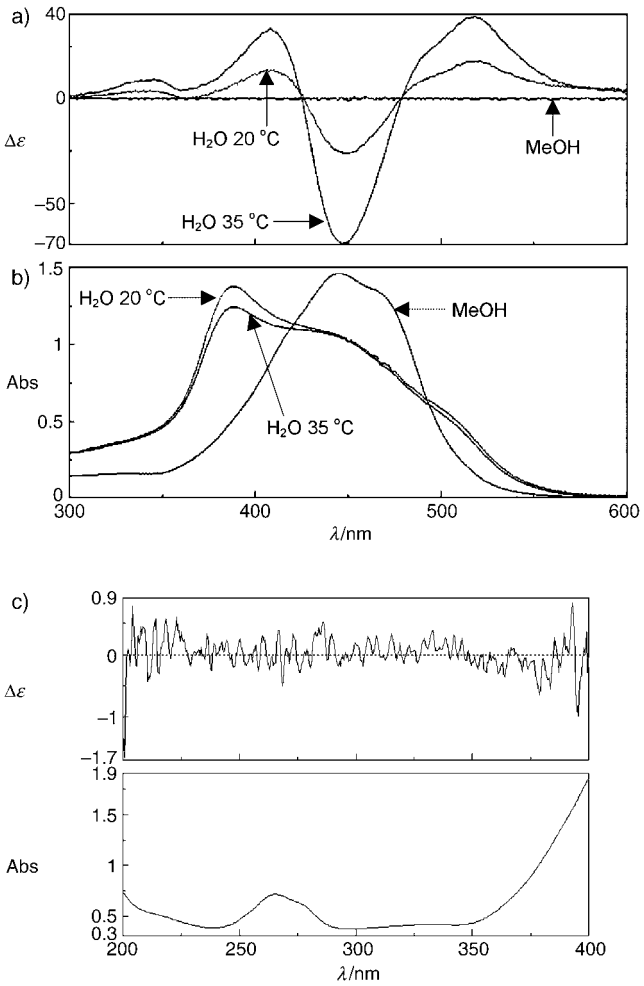
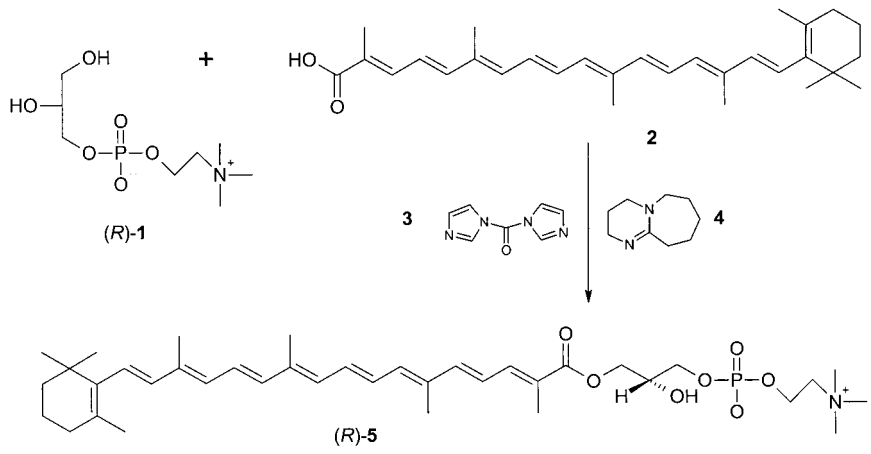


Figure 1. CD spectra (a) and absorptions spectra (b) of (*R*)-**5** in the range 300–600 nm and c) CD and absorption spectra of (*R*)-**5** in the range 200–400 nm; path length = 1 cm, $c = 2 \times 10^{-5}$ M (critical micelle concentration of (*R*)-**5**: $c_M = 1.5 \times 10^{-3}$ M).[13]



Scheme 1.

In water, the amphiphilic carotenoylphospholipid (*R*)-**5** forms clear, orange-colored dispersions, mostly of aggregates with an average size of 6 nm.[13,14] The strong absorption band of the monomeric solution observed at 445 nm in methanol (π–π* transitions of the polyene chain, Figure 1b), splits in water into two exciton bands, a prominent signal at 380 nm in the H-aggregate and a slightly visible shoulder at 510 nm in the J-aggregate region.[36,37] The association of

monomers into aggregates accelerated at higher temperature, the intensity of the Cotton effect increased when the sample was left to stand at 35 °C (Figure 1a). While the flat line in the CD spectrum convincingly proves the lack of Cotton effects in the molecular solution of (*R*)-**5** (in methanol) the CD spectrum of (*R*)-**5** in water showed strong Cotton effects (Figure 1a), a positive signal at 410 nm, a strong negative band in the absorption region of the polyene chain (445 nm), and again a positive band at 520 nm. Since simply water was added to optically inactive (*R*)-**5** the obtained Cotton effects can only be generated from induced optical activity, originating from a chiral association of (*R*)-**5** monomers. In principle, the observed optical activity of the aggregates could arise from a handed orientation of all monomers in a possibly existing unilamellar vesicle.[13,14] However, it is also possible that a few monomers first associate to give small primary units, which then constitute the supramolecular structure. To determine the origin of EOA in the aggregates, we tried to simulate the absorption and CD spectra. At first sight, the experimental CD spectrum seems to be a mixture of blue-shifted negative and red-shifted positive exciton couplets. The most simple model that explains such spectral behavior would be a tetramer with both right- and left-handed overlay angles. Molecular mechanics calculations were performed using the Sybyl 6.6 program[38] to find a minimum-energy conformation. The calculated tetramer (Figure 2) appeared to be in accordance with the spectral data.

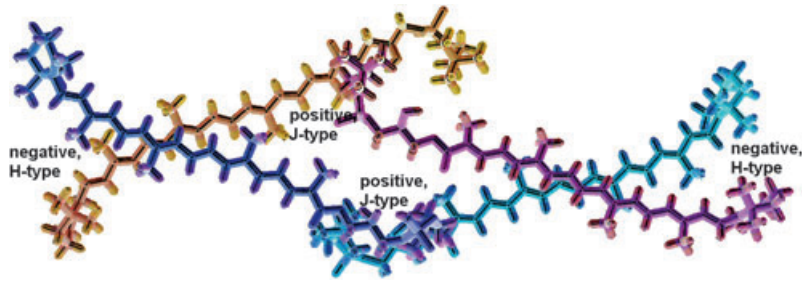


Figure 2. The simplest unit with both negative H-type and positive J-type arrangements.

However, the tetramer arrangement did not fully account for the exciton interactions according to the excitation model of Buss et al.[39] To account for these interactions two tetrameric substructures were combined and optimized using molecular dynamics (MD) and force-field-CVFF[40–42] calculations in the Discover program.[43] The UV/Vis and CD spectra of the aggregates could then be simulated with the carotenoid acid **2** in the AM1-model by applying a configuration interaction (CI) with three occupied and two unoccupied MOs and considering singly as well as doubly excited configurations. The lowest excitation was calculated at 519 nm with a transition moment of 2.74 D. These values were then used within the excitation model by employing the dipole–dipole approximation. The computation resulted finally in a structure (Figure 3), which satisfactorily explains the recorded spectra (see Figure 4).

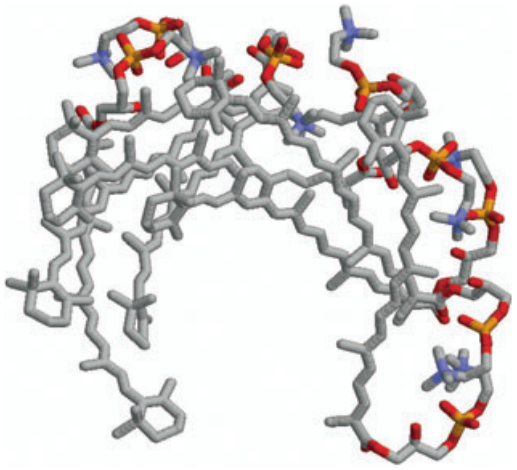


Figure 3. Optically active *P*-oligomer unit, built from eight optically inactive (*R*)-**5** monomers.

We conclude therefore that octamers form the primary units leading to the observed chiroptical absorption.[44] The configuration of these octamers is determined by the stereogenic center in the hydrophilic part of monomeric (*R*)-**5**. The CD spectra express supramolecular chirality of the aggregates resulting from a helical *P* orientation of the polyene chain in the calculated oligomer (Figure 3).

Basic units in aggregates have rarely been reported,[45,46] for example, the monomers of the mentioned styrylthiophene phospolipid create a tetramer unit.[12] The formation of aggregation units is consistent with the observation of quantized aggregation numbers (AN),[47] where the total aggregation number can only be a multiple of monomers in the primary aggregation unit.

Aggregates of chiral carotenoids often show characteristic exciton couplets in the CD spectra, which are caused by chromophores overlapping in specific, ordered arrangements.[48–51] Such ordered arrangements are not encountered in the oligomer discussed here. The inadequate agreement of the maxima, minima, and zero-point crossings of the CD spectrum with that of the absorption spectrum reflects the rather irregular structure of the calculated oligomer. Further, it follows from Figure 3 that no defined H- or J-arrangements exist in the depicted octamer. The interchromophoric geometry of the molecules in the octamer causes a shift of the absorption maxima to shorter as well as to longer wavelengths.

A basic unit, such as the selected octamer, cannot exist as an independent entity in water, since the polar and nonpolar groups are oriented in an unfavorable way. In addition, dynamic light scattering measurements did not detect oligomeric particles corresponding in size to the association of

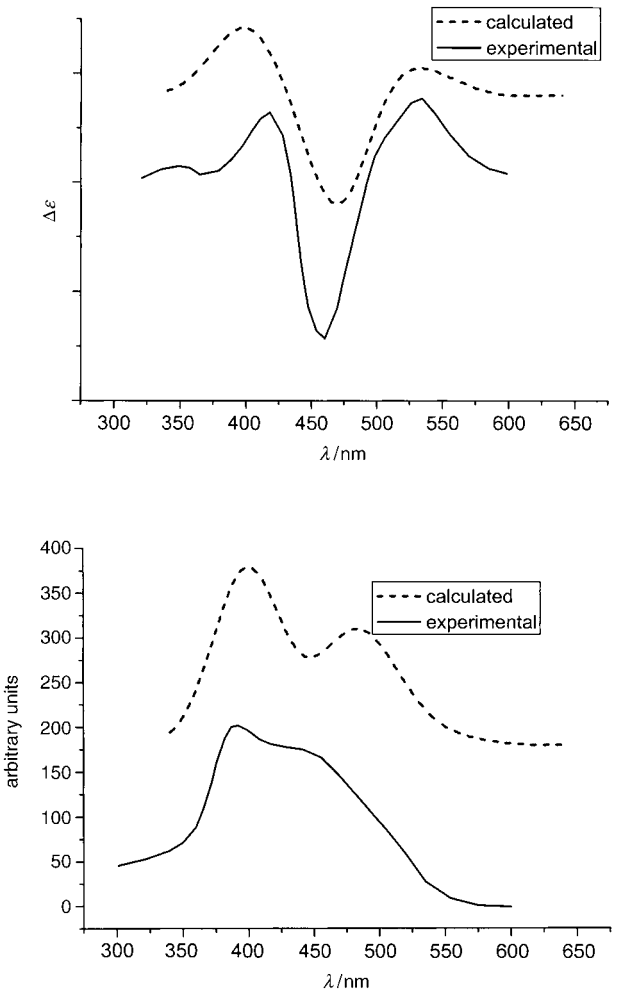


Figure 4. Calculated and experimental CD (top) and absorption (bottom) spectra of (*R*)-**5**; the calculated spectra are shifted by +40 nm for better adjustment.

eight monomers.[13,14] Therefore, we define the oligomer unit as the lowest spatial extent of monomer association that displays all the spectroscopic and chiroptical properties of the aggregate. Such basic units in aggregates could possibly be compared with elementary (Bravais) units in crystals.

Aggregate formation is sensitive to subtle experimental conditions. Besides the absorption at 380 nm, (Figure 1b), aggregate dispersions absorbing at 390 or 400 nm were sometimes observed suggesting the possible presence of other oligomer structures in these higher absorbing aggregates.[14]

The few known Cotton effects of glycerophospholipid aggregates arise from the ester carbonyl group located in the hydrophilic part of molecules at the exterior of the aggregates.[22,23] In contrast, the Cotton effects of (*R*)-**5** are formed by the hydrophobic polyene chromophores and correspond to molecular interactions inside the aggregates (micelles) or inside the membrane (lamellar vesicles). Aggregates of (*R*)-**5** should possess an enantiomorphous membrane with the potential to differentiate enantiomeric reactants. In a preliminary experiment, the membrane-opening protein melittin[52] did not disrupt the membrane of (*R*)-**5**, as demonstrated by the absence of energy and electron transfer in flash photolysis experiments.

Conclusion

Reviewing our experimental results and molecular calculations, we conclude that the highly unsaturated, optically inactive glycerophospholipid (*R*)-**5** forms enantiomeric primary units of moderate size in water: an octameric oligomer may represent the simplest repeating unit, satisfactorily expressing absorption properties and supramolecular EOA.

It appears reasonable to hypothesize that other (crypto)optically active glycerolipid monomers also may associate to give small, enantiomeric units within supramolecular assemblies.

Experimental Section

The product [(*R*)-5] was isolated by means of flash chromatography (Silica 60 A 40–63 mm, SDS). The column was prepared with $CHCl_3$, the product dissolved in $CHCl_3$ and eluted with $CHCl_3/CH_3OH$ (80/20), gradually increasing the amount of water and methanol to $CHCl_3/CH_3OH/H_2O$ (40:50:10). Smaller amounts were purified on analytical DC plates (Silika 60 F254, Merck) with $CHCl_3/CH_3OH/H_2O$ (40:50:10) as eluent. The product yield was determined from the UV/Vis spectrum.[53]

(*R*)-1-(β-Apo-8′-carotenoyl)-3-glycerophosphocholin [(*R*)-5]: The intermediate carotenoylimidazole was synthesized by reacting β-apo-8′-carotenoic acid (**2**;[16] 77 mg, 0.18 mmol) with 1,1′-carbonyldiimidazole (**3**) (292 mg, 1.8 mmol) dissolved in $CHCl_3$ (10 mL). The reaction mixture was stirred under nitrogen (20 °C, 4 h). *sn*-1-Glycerophosphocholine ((*R*)-**1** ; 100 mg, 0.39 mmol), $[\alpha_D^{18}] = -2.5°$, $c = 0{,}05\ g\,mL^{-1}$ H_2O, $p = 0.88$, ($[\alpha_D^{19}] = -2.84°$, $c = 0.088\ g\,mL^{-1}$ H_2O[54]) and 1,8-diazabicyclo[5.4.0]undec-7-ene (**4**) (119 mg, 0.78 mmol), dissolved in DMSO, were added to the solution, and the reaction mixture was stirred under nitrogen (40 °C, 24 h). The solution was washed with saturated NaCl (aq) to remove most of the DMSO and then dried over Na_2SO_4. After evaporation of the solvent under reduced pressure chromatographic work-up gave (*R*)-**5** (5 mg, 4%). UV/Vis in MeOH and H_2O: see Figure 1b, c. CD in MeOH and H_2O: see Figure 1a, c. The ^1H, ^{13}C, and ^{31}P NMR spectra are given in reference [55]. No isomerization by acyl migration was detected.

Attempt at membrane disruption: Phospholipid (*R*)-**5** in water was irradiated with rose bengal as senzitizer. The transient absorption spectra of carotenoid triplets (^{3}car) or of carotenoid cation radicals (car$^{\bullet+}$) could not be observed. Melittin (Bachem) was added and, after few minutes, the solution was irradiated. Again, no energy or electron transfer occurred. The photophysics of (*R*)-**5** and other water-dispersible carotenoids will be published elsewhere.

Description of the calculation methods:

Calculation of the tetrameric structure: Molecular mechanics (MM) calculations were performed by using the Sybyl 6.6 program[38] on a Silicon Graphics Octane workstation with an Irix 6.5 operating system. The MM calculations were based on MMFF94 force field,[56] in which energy minimization was applied by the conjugate gradient technique with 0.001 kcal mol^{-1} Å^{-1} gradient. Tetramers were built up from energy-minimized monomers using the dock command of Sybyl.[38]

Calculation of the octameric structure: Molecular dynamics (MD) calculations were performed by using the Discover 2.9.7 program[43] on an SGI ORIGIN 2000 mainframe with an Irix 5.1 operating system. The applied force filed was CVFF.[40–42] To determine the conformational space, the

starting geometry was first equilibrated at 300 K for 500 ps followed by slowly cooling to 100 K. The final geometry was obtained by applying the steepest descent technique for 1000 steps and finally by applying the conjugate gradient with a 0.001 kcal mol^{-1} Å^{-1} gradient. The AM1 (MOPAC 93, Fujitsu Limited, Pentium III, 700 MHz, Windows NT4.0) method with a CI of the three highest occupied and the two lowest unoccupied MOs was used to calculate the excitation energies and transition moments. The individual transition moments were superimposed on the individual molecules within the aggregates. Applying the principle of dipole–dipole interactions,[57] an energy matrix was constructed and diagonalized as usual. In this way the eigenvalues and, thus, the excitation energies of the aggregates and the corresponding oscillatory and rotatory strengths were obtained.

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