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Chiral Amplification in Nature: Cell-extracted Chiral Carotenoid Microcrystals Studied via RROA of Model Systems

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Abstract: Intriguingly, we have observed that carotenoid microcrystals, extracted from cells of carrot roots and built from 95% achiral β-carotene, exhibit a very intense chiroptical (ECD and ROA) signal. This serendipitous discovery of preferential chirality of crystalline aggregates, composed mostly from achiral building blocks is a newly observed phenomenon in nature and with high probability may be related to asymmetric information transfer from chiral seeds (small amount of α-carotene or lutein) present in carrot cells. To confirm this hypothesis, we have synthesized several model aggregates built from various achiral and chiral carotenoids. Because of the sergeant-and-soldier behaviour, a small number of chiral sergeant (a-carotene or astaxanthin) forced achiral soldier molecules (β - or 11,11'-d₂- β -carotene) to form jointly supramolecular assemblies of induced chirality. Chiral amplification observed in these model systems proved that chiral microcrystals appearing in nature might be composed predominantly from achiral building blocks and their supramolecular chirality might be the result of cocrystallization of chiral and achiral analogues, due to their proximity in cell compartments.

Carotenoid microcrystals are formed in crystalline chromoplasts of carrot root,^[1,2] where a needle-like, rhomboidal, and helical types can be found.^[3,4] The size of those crystalline aggregates is usually of a few µm up to 20 µm, what was determined by means of the AFM technique.^[3] The predominant component of natural microcrystals is achiral β-carotene (βC) accompanied by a small contribution of chiral α-carotene (αC) and lutein.^[3–5] Moreover βC molecules constitute *ca.* 95% of total carotenoids content of measured specious.^[6] Despite the achiral composition, microcrystals reveal natural chirality at the supramolecular level. Here, we present the study of carotenoid microcrystals, as well as several model systems using Electronic Circular Dichroism (ECD) and Raman Optical Activity (ROA). We want to prove the hypothesis that microcrystals' optical activity might be driven by the process of chirality induction, leading to a chiral signal

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Supporting information for this article is given via a link at the end of the document.

amplification. To explore this phenomenon, we prepared model aggregates built from the combination of chiral and achiral carotenoids, following a sergeant-and-soldiers rule.^[7,8] According to the effect, a small number of chiral units (sergeants) forces a large number of achiral ones (soldiers) to adopt a supramolecular structure and to display an intense chiral signal.^[9,10] This kind of chirality amplification was observed for other supramolecular systems^[11-13] like foldamers,^[14] helical nanotubes and columns,^[15,16] as well as for polymers and copolymers.^[17-19] The chiral sense of studied here model aggregates comes from the chirality transfer between: chiral aC or astaxanthin (AXT) molecules and achiral βC components, which together form a mixed supramolecular chiral systems exhibiting the resonance enhancement of ROA (RROA). On the other hand, aggregates composed of only chiral carotenoids were studied by us before.^[20-23] Those assemblies also show a strong optical activity, generated by a non-symmetric layout of molecules through the non-covalent interactions (π – π stacking, hydrogen bonding, hydrophobic effects). In detail, the chiral information from a carotenoid chiral centre is imposed to the whole system containing a chromophore (polyene chain) in the chiral conjugated arrangement. The process of carotenoid aggregation enables observation of a significant enhancement in the ROA scattering, called by us the Aggregation-Induced (AIRROA).^[20-23] Resonance Raman Optical Activity Nevertheless, ROA spectroscopy is hampered by its low sensitivity, because only one photon in a billion gives rise to the ROA effect.^[24] The AIRROA phenomenon causes a substantial increase in the ROA intensity due to the aggregation of supramolecular chiral structures, of a resonantly enhanced chiroptical signal. The high vibrational specificity and the enhanced sensitivity of AIRROA make it a unique tool to explore chirality of supramolecular model aggregates and natural carotenoid microcrystals. Amplification of chirality in momentary asymmetric systems is considered a key phenomenon in understanding the origin of homochirality of biological macromolecules.^[25] This model study on chiral amplification in dynamic supramolecular systems proves that chiral appear in nature purely in microcrystals may the physicochemical process of chiral induction due to the proximity of achiral and chiral analogues in the cell environment.

Here we focus on crystalline aggregates released from carrot cells using an extraction procedure described in the Supporting Information. Microscopic images of helical, rhomboidal, and needle-like microcrystals are presented in Figure 1. The ECD spectrum reveals quite strong negative–positive bands (going from longer to shorter wavelengths), known as bisignate Cotton effects, with a clearly accentuated vibrational structure, also seen in the UV-Vis spectrum and typical for carotenoids without conjugated carbonyl groups (like β C).^[26–28] Because of the coincidence of the ROA excitation laser line (532 nm) with an absorption of the main chromophore, carotenoid microcrystals show an enhanced and monosignate RROA spectrum (Figure 1), opposite in sign to the ECD

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Figure 1. Optical images of microcrystal isolated from carrot cells. The size of aggregates is of a few µm up to 20 µm. The ECD spectrum shows strong, negative/positive chiral signal (from -30 to 15 mdeg) in the range of the UV-Vis spectrum. The ROA laser (green dashed line, 532 nm) coincides with the microcrystal absorption, thus the resonance Raman (dotted line) and ROA (solid line) spectra were recorded. A, B, C symbols represent inserts of Raman and RROA bands in the appropriate spectrum range.

transition in the excitation range, in agreement with Nafie's theory of the Single Electronic State (SES) limit.^[29] We observe characteristic RROA bands due to typical carotenoid vibrations, i.e.: v_1 at 1519 cm⁻¹, v_2 at 1159 cm⁻¹, v_3 at 1009 cm⁻¹, and v_4 at 956 cm⁻¹, which are assigned to the C=C stretching, C-C stretching, the in-plane CH₃ rocking, and the C-H out-of-plane wagging motions coupled with the C=C out-of-plane torsion modes, respectively.^[30,31] In addition, the RROA shoulder localized at 967 cm⁻¹ is associated with the symmetric C-H and C out-of-plane wagging^[32] with a small contribution due to β rings' deformations^[31] and the C-C stretching vibrations.^[33] The Raman imaging study reported that the position of the v_1 band at 1519 cm⁻¹, as well as the v_1/v_2 ratio of integral intensities (1.005) is specific for the helical crystalline aggregates and the presence of β C in crystal content.^[3,4] We claim, that the presence of those features in the RROA spectrum is related with the induced chiral information from β C. In addition, the band at 956 cm⁻¹ is characteristic for both the needle-like and rhomboidal microcrystals. (A detailed spectral analysis is given in the Supporting Information).

 βC molecules aggregate in the hydrated organic environment, which is seen by the shift of the UV-Vis band, as well as by the frequency decrease of the v_1 Raman band, in comparison to the monomer spectrum (Figure 2, left). Moreover, βC has a tendency to form J-type aggregates $^{[34]}$ (a red-shift of an absorption band) due to the methylated and twisted cyclohexene rings, as well as the lack of OH groups. For flatter carotenoids (for example lutein), the H-type aggregates are favourable, in which π,π -stacking and hydrogen bonds are stronger. $^{[2,35,36]}$ βC assemblies do not give chiroptical signals, as seen in the ECD and RROA spectra (Figure 2, left). Thus, the chirality of the supramolecular system, and its lack results in the formation of racemic aggregates from a macroscopic perspective. $^{[9]}$ In

contrast to BC, a model system built from the combination of achiral βC (80%) and chiral αC (20%) shows a chiral signal in the ECD and RROA spectra (Figure 2, right). To prove the induction of BC chirality at the supramolecular level a few strong spectral features can be pointed out: 1/ the outlined vibrational ECD/UV-Vis structure; [26-28] 2/ the value of Raman and RROA v_1/v_2 ratio close to 1 (0.95);^[3] 3/ the position of v_1 Raman and RROA band at 1520 cm⁻¹, i.e. shifted. Similar to carotenoid microcrystals, we observe the RROA band at 965 cm⁻¹ due to the hydrogen-out-of-plane (HOOP) motions. The HOOP mode is recognized as a sensitive marker of distortion of the polyene chain.^[37,38] Therefore, its occurrence is most likely related to the bending of the carotenoid chain in the $\beta C/\alpha C$ model, which probably comes from aC, whose structure is more bent (Figure S2). Overall, it is important to note that the non-chiral (UV-Vis and Raman) spectra of the achiral (β C) and chiral (β C/ α C) systems are strikingly similar, confirming that both aggregates are structurally similar. In addition, the Raman spectra of both aggregates resemble closely the ROA spectrum of the $\beta C/\alpha C$ system, showing that the chiral signal is predominantly related to BC.

To exclude further the possibility that the chiral signal recorded for the $\beta C/\alpha C$ model results solely from αC molecules, we investigated the concentration-dependent behavior of αC in a water–acetone solution. Figure 3 (left) shows clearly that αC aggregates at such small concentrations do not give a noticeable chiroptical signal. In particular, the αC assemblies obtained at the concentration used for the $\beta C/\alpha C$ system also do not give any ECD spectrum (cyan line). On the other hand, the ECD signal is already observed for the $\beta C/\alpha C$ 95:5% system, where a chiral αC constitutes only 5% of the total carotenoid content, i.e. at the concentration of ca. $4 \cdot 10^{-7}$ M (Figure 3, right, violet line).

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Figure 2. Comparison of β C aggregate (orange) and β C/ α C system (pink). The UV-Vis spectrum of β C aggregate is red shifted, but the ECD does not show a chiral signal. Contrarily, the ECD of β C/ α C system presents intense positive/negative bands (from 20 to -20 mdeg), stable after 24 hours (pink, dashed line). In like manner, β C aggregate does not give the RROA spectrum, but β C/ α C model does (the negative profile of RROA). The Raman spectra of both systems (dotted line) and monomer (black, dotted line) are also presented. A, B show inserts of the Raman and RROA profiles in the appropriate spectrum range. * represents Raman acetone bands.



Figure 3. Left panel: The concentration dependent ECD and UV-Vis spectra of α C in the aggregated form. Violet colour means the lowest concentration of α C used in the preparation of β C: α C95:5% mixed aggregate (panel right). Cyan colour represents the α C concentration used in the formation of β C/ α C model system (Figure 2). Right panel: ECD and UV-Vis spectra of mixed aggregate consisting of 95% β C and 5% α C.

To verify the induced chirality of BC in aggregated mixed systems, we built another model using (3S,3'S)-astaxanthin (AXT) as a chiral sergeant and compared it with a system containing solely AXT. As presented in Figure 4 (left), the βC/AXT model system also shows a strong optical activity. The outlined ECD vibrational structure (maxima located at 518, 485, 454 nm), the position of the RROA v_1 band (1519 cm⁻¹) and the ratio between integral intensities of v_1/v_2 (1.12), may be recognized as typical for β C in the aggregated form and may be considered as a chiral response of βC at the supramolecular level. Moreover, these spectral features are significantly different compared with single aggregated AXT molecules (Figure 4, right). The red-shift of the UV-Vis band, and the frequency decrease of the Raman C=C mode (1525 \rightarrow 1521 cm⁻¹) are typical for the J-type arrangement, which has been studied in detail before.^[22,23] To confirm that the chiral signal of the β C/AXT model is not a result of the chirality of AXT itself, we highlight some spectral differences between these two aggregates. For the AXT J aggregate the v_1/v_2 intensity ratio equals 1.72, as well as the 1217 cm⁻¹ RROA band, assigned to the C-C stretching combined with the ring methylene twist vibration,[31-33] is significantly weaker compared with this band (at 1211 cm⁻¹) in the β C/AXT model spectrum. Neither the RROA profile exhibits the HOOP band (v₄), which implies the distortion of the polyene chain. Its absence may be connected with the lower deformation of the carotenoid chain in the 'looser' J aggregate, which consists of several layers of monomers, twisted with respect to those above and below.^[2,36,39]

To confirm ultimately the presence of βC inside the supramolecular structures of mixed aggregates, we considered another $\beta C/AXT$ model, formed using synthesized de novo 11,11'-d₂- β -carotene derivative (βC -d, for synthesis refer to the Supporting Information), and keeping the same AXT sergeant and the procedure of aggregate preparation. As a result of deuteration, in the βC -d/AXT system we can observe (Figure 5): 1/ the opposite ECD profile that corresponds to the opposite chirality and the twisting direction (handedness) of supramolecular structure; 2/ the RROA/Raman frequency shifts of v₁ (1519 \rightarrow 1514 cm⁻¹) and v₂ (1159 \rightarrow 1162 cm⁻¹) bands; 3/ the presence of the HOOP band at 970 cm⁻¹ in the RROA spectrum (Figure 5C).



Figure 4. Comparison of the β C/AXT model (green) and the AXT J aggregate (blue). The UV-Vis spectra of both systems are red shifted. The ECD spectra show strong optical activity, however different profiles confirm the formation of different aggregates' types. Both systems give enhanced and negative RROA spectra, although some different features can be pointed: the position of C=C stretching band (1519, 1515 cm⁻¹) or the integral intensity ratio of two main bands (1519/1159 cm⁻¹ and 1515/1161 cm⁻¹). Raman spectra of both systems, as well as monomer (black) are also presented. * represents Raman acetone bands.

Being structurally sensitive, the HOOP band^[37,38] can be considered as evidence of the presence of deuterated (more bent) BC molecules in the supramolecular mixed arrangement, which gives a chiral response. Moreover, an increase of the HOOP band intensity can also be seen in the Raman spectra of the BC monomer and its aggregate before, as well as after deuteration (Supporting Information, Figure S5). However, based on the vibrational analysis of 11,12-dideuterio retinal,^[40] it can be seen that the HOOP band is enhanced for deuterated derivatives, which confirms a substantial deuterium-induced change in the molecular structure of BC-d compared with BC. Strikingly, this change is reflected not only in the BC-d Raman spectrum, but also in the ROA spectrum of β C-d/AXT aggregate. Independently of its origin, the band at 970 cm⁻¹ can be treated as an internal marker of BC-d, and therefore, its presence in the ROA BC-d/AXT spectrum confirms that BC-d becomes optically active in the mixed system.

On the other hand, the RROA spectrum of the β C-d/AXT model reveals three other spectral features, which are shared with the former systems. First, the value of the RROA ratio between v_1/v_2 is 1.32. Second, the integral intensity ratio between RROA bands at 1214 and 1195 cm⁻¹ of the β C-d/AXT

model equals 0.97. Finally, the presence of the RROA combination band at 2487 cm⁻¹ originating from the fundamental of vibrations located at 1514 and 970 cm⁻¹.

Observation of a strong chiroptical signal from carotenoid microcrystals extracted from the cells of carrot roots and built mostly (95%) from achiral BC fits into the bigger picture of of chirality amplification in carotenoid supramolecular assemblies. Because of the unique capabilities of the AIRROA we were able to demonstrate in several model systems that chiral amplification in a sergeant-and-soldier fashion is a universal phenomenon occurring for various carotenoid pairs of sergeants and soldiers. Above all, the study demonstrates that carrot microcrystals in carrot cells gain chirality in a purely physicochemical process of chiral amplification occurring most likely due to the proximity of sergeants and soldiers (in this case αC and βC , respectively) in crystalline chromoplasts. This work shows the potential of chiroptical methods in studying natural biological systems of amplified chirality that brings hope for a better understanding of the origin of chirality in biological macromolecules.



Figure 5. Comparison of the β C-d/AXT model (violet solid line) and the β C/AXT aggregate (dotted green line) in order to show the effect of deuteration. The ECD spectra of both systems have similar, but opposite profiles. The UV-Vis spectra of both models are red shifted, what confirms the aggregation process. Subsequently, both aggregates give the enhanced and opposite RROA spectra. Main changes due to deuteration: the frequency shift of main bands (1519 \rightarrow 1514 cm⁻¹, 1159 \rightarrow 1162 cm⁻¹), the presence of the HOOP RROA band at 970 cm⁻¹ (insert C) and the combination band at 2487 cm⁻¹ (insert A). Raman spectra of both systems, as well as monomer (black) are also presented. * represents Raman acetone bands.

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Keywords: carotenoid microcrystals • chirality induction • resonance Raman Optical Activity • supramolecular chirality • sergeant-and-soldiers effect

Conflict of interest

The authors declare no conflict of interest.

- [1] J. E. Kim, K. H. Rensing, C. J. Douglas, K. M. Cheng, *Planta* 2010, 231, 549–558.
- [2] S. Köhn, H. Kolbe, M. Korger, C. Köpsel, B. Mayer, H. Auweter, E. Lüddecke, H. Bettermann, H.-D. Martin, in *Carotenoids SE - 5* (Eds.: G. Britton, S. Liaaen-Jensen, H. Pfander), Birkhäuser Basel, 2008, pp. 53–98.
- [3] A. Rygula, T. Oleszkiewicz, E. Grzebelus, M. Z. Pacia, M. Baranska, R. Baranski, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 2018, 197, 47–55.
- M. Roman, K. M. Marzec, E. Grzebelus, P. W. Simon, M. Baranska, R. Baranski, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* 2015, 136, 1395–1400.
- [5] M. Baranska, R. Baranski, E. Grzebelus, M. Roman, *Vib. Spectrosc.* 2011, 56, 166–169.
- [6] T. Oleszkiewicz, M. Klimek-Chodacka, A. Milewska-Hendel, M. Zubko, D. Stróż, E. Kurczyńska, A. Boba, J. Szopa, R. Baranski, *Planta* **2018**, *248*, 1455–1471.
- [7] M. M. Green, N. C. Peterson, T. Sato, A. Teramoto, R. Cook, S. Lifson, *Science (80-.).* **1995**, *268*, 1860 LP-1866.
- [8] M. M. Green, M. P. Reidy, R. D. Johnson, G. Darling, D. J. O'Leary,
 G. Willson, J. Am. Chem. Soc. 1989, 111, 6452–6454.
- [9] M. Liu, L. Zhang, T. Wang, *Chem. Rev.* **2015**, *115*, 7304–7397.
- [10] G. Pescitelli, L. Di Bari, N. Berova, Chem. Soc. Rev. 2014, 43, 5211–5233.
- [11] A. L. Hofacker, J. Parquette, Amplification of Local Chirality within a Folded Dendrimer. An Intramolecular "sergeants and Soldiers" Experiment, 2010.
- [12] V. K. Praveen, S. S. Babu, C. Vijayakumar, R. Varghese, A. Ajayaghosh, *Bull. Chem. Soc. Jpn.* **2008**, *81*, 1196–1211.
- [13] H. Engelkamp, S. Middelbeek, R. J. M., Nolte, Science (80-.). 1999, 284, 785 LP-788.
- [14] C. Dolain, H. Jiang, J.-M. Léger, P. Guionneau, I. Huc, J. Am. Chem. Soc. 2005, 127, 12943–12951.
- [15] T. W. Anderson, J. K. M. Sanders, G. D. Pantoş, Org. Biomol. Chem. 2010, 8, 4274–4280.
- [16] L. Brunsfeld, B. G. G. Lohmeijer, J. A. J. M. Vekemans, E. W. Meijer, J. Incl. Phenom. Macrocycl. Chem. 2001, 41, 61–64.
- [17] J. Bergueiro, F. Freire, E. P. Wendler, J. M. Seco, E. Quiñoá, R. Riguera, *Chem. Sci.* 2014, 5, 2170–2176.
- [18] E. Yashima, K. Maeda, T. Nishimura, Chem. A Eur. J. 2004, 10, 42–51.
- [19] J. van Gestel, Macromolecules 2004, 37, 3894–3898.
- [20] M. Dudek, G. Zajac, A. Kaczor, M. Baranska, J. Raman Spectrosc. 2017, 48, 673–679.
- [21] G. Zajac, J. Lasota, M. Dudek, A. Kaczor, M. Baranska, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 2017, 173, 356– 360
- [22] M. Dudek, G. Zajac, A. Kaczor, M. Baranska, J. Phys. Chem. B 2016, 120, 7807–7814.

- [23] G. Zajac, A. Kaczor, A. Pallares Zazo, J. Mlynarski, M. Dudek, M. Baranska, J. Phys. Chem. B 2016, 120, 4028–33.
- [24] L. D. Barron *, L. Hecht, I. H. McColl, E. W. Blanch, *Mol. Phys.* 2004, 102, 731–744.
- [25] A. R. A. Palmans, E. W. Meijer, Angew. Chemie Int. Ed. 2007, 46, 8948–8968.
- [26] C. Wang, C. J. Berg, C. C. Hsu, B. A. Merrill, M. J. Tauber, J. Phys. Chem. B 2012, 116, 10617–10630.
- [27] D. Zigmantas, R. G. Hiller, F. P. Sharples, H. a. Frank, V. Sundstrom, T. Polivka, *Phys. Chem. Chem. Phys.* 2004, 6, 3009.
- [28] H. A. Frank, J. A. Bautista, J. Josue, Z. Pendon, R. G. Hiller, F. P. Sharples, D. Gosztola, M. R. Wasielewski, *J. Phys. Chem. B* 2000, 104, 4569–4577.
- [29] L. A. Nafie, *Chem. Phys.* **1996**, *205*, 309–322.
- [30] J. C. Dobrowolski, in *Carotenoids*, John Wiley & Sons, Ltd, **2016**, pp. 75–102.
- [31] M. Macernis, D. Galzerano, J. Sulskus, E. Kish, Y.-H. Kim, S. Koo, L. Valkunas, B. Robert, J. Phys. Chem. A 2015, 119, 56–66.
- [32] C. J. Berg, A. M. LaFountain, R. O. Prum, H. A. Frank, M. J. Tauber, Arch. Biochem. Biophys. 2013, 539, 142–155.
- [33] N. Tschirner, M. Schenderlein, K. Brose, E. Schlodder, M. A. Mroginski, C. Thomsen, P. Hildebrandt, *Phys. Chem. Chem. Phys.* 2009, *11*, 11471–11478.
- [34] J. Hempel, C. N. Schädle, S. Leptihn, R. Carle, R. M. Schweiggert, J. Photochem. Photobiol. A Chem. 2016, 317, 161–174.
- [35] H. H. Billsten, V. Sundström, T. Polívka, J. Phys. Chem. A 2005, 109, 1521–1529.
- [36] F. Zsila, Z. Bikádi, Z. Keresztes, J. Deli, M. Simonyi, J. Phys. Chem. B 2001, 105, 9413–9421.
- [37] T. Fujisawa, R. L. Leverenz, M. Nagamine, C. A. Kerfeld, M. Unno, J. Am. Chem. Soc. 2017, 139, 10456–10460.
- [38] G. Eyring, B. Curry, A. Broek, J. Lugtenburg, R. Mathies, Biochemistry 1982, 21, 384–393.
- [39] F. Zsila, Z. Bikádi, J. Deli, M. Simonyi, *Chirality* **2001**, *13*, 446–453.
- [40] B. Curry, A. Broek, J. Lugtenburg, R. Mathies, J. Am. Chem. Soc.
 1982, 104, 5274–5286.

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Into the chirality induction: Natural microcrystals and model aggregates are consist mainly of achiral βcarotene accompanied by a small contribution of chiral carotenoids. According to the sergeant-andsoldiers effect, a small number of chiral units imposes a large number of achiral ones leadning to the amplification of a chiral signal, which can be observed by means Electronic Dichroism Circular (ECD) and resonance Raman Optical Activity (RROA).



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