Ascorbyl-oleate: a Bioconjugate Antioxidant Lipid

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Abstract: Ascorbyl-6-O-oleate (Asc-OL) was synthesized enzimatically and studied in the pure state and in aqueous dispersions. SAXS, DSX, FTIR experiments and antioxidant tests were conducted in order to characterize the compound and to evaluate its reducing properties. Asc-OL is a bioconjugate molecule, in fact it combines the main physico-chemical features of the two parent molecules, i.e. the redox and acidic properties of Vitamin C and the fluid state of the oleic acid tail. The DPPH and ethyl linoleate tests confirmed the excellent radical scavenging activities of Asc-OL, that turns out to be a promising candidate for the stabilization, transport and protection against radical attack of valuable hydrophobic active ingredients used in food and pharmaceutical formulations.

Introduction

L-ascorbic acid is one of the most potent antioxidant water soluble molecules. As a radical in its own right, it scavenges harmful species that attack and damage biomolecules central to crucial processes in the life cycle of an organism. Examples are proteins, nucleic acids and some unsaturated biomembrane lipids. Our world is exposed to worsening environmental conditions. A few of these are: a significant increase in the concentration of radical species in the atmosphere (particularly nitrogen oxides); the seasonal partial loss of the ozone layer; and increasing ultraviolet radiation.[1,2] Further, global warming might speed up radical-based endothermic processes. Such considerations reinforce the desirability of better protecting terrestrial life from free radical attack via the deployment of antioxidant species.[3-5]

Cell protection against singlet oxygen damage can be achieved by several quenching molecules. They include ascorbic acid, carotenoids and phenols and notably the tocopherols.[6] Vitamin C is a weak singlet oxygen quencher. With a standard reduction potential of +0.23 V, it is in fact a better single-electron reducing agent than tocopherol. Amphiphilic hydrocarbon based derivatives of vitamin C form supramolecular nano-self-assembled aggregates in water. Their physico-chemical properties depend on the length and saturation of the hydrocarbon moieties.[7]

Within such nanostructures, L-ascorbic acid derivatives can protect degradable hydrophobic species (especially unsaturated fats or vitamins), by dispersing them in the hydrophobic core. At the same time there is a synergy, with the ascorbic acid polar heads exposed to the water phase able to carry out their anti-radical work.[8]

We have chosen oleic acid as the ascorbic acid partner. It is an omega-9 fatty acid, abbreviated with a lipid number of 18:1 cis-9. The main uses of oleic acid and oleates derive from their emulsifying properties, particularly in food formulations and in pharmaceuticals.[9] Oleic acid is an important supplement in human diet as a constituent of animal fats and vegetable oils. It is liquid at room temperature, with melting point ~ 13° C. With small amounts of salts such as KCl, oleic acid and/or oleate aqueous dispersions can form viscoelastic systems (VES). These have attracted much attention due to their versatility in a variety of high-tech and everyday applications.[10,11] Variation of solution conditions like pH, surfactant and salt concentration, ion pair, temperature, and solvent compostion, allow VES amphiphiles to form a rich diversity of nanostructures. These are determined by a surfactant packing parameter (*p*).[12-14]

At “high” concentrations, the nanostructures take the form of an entangled network. This gives rise to a viscoelastic behaviour that is reminiscent of that of flexible polymer solutions.[15,16] VES systems can also be used to produce “smart” materials. Their properties can be controlled reversibly in response to an external stimulus. The stimuli include changes in temperature, electrical or magnetic fields, pH, UV-visible irradiation, ionic or metallic interactions.[17,18] The formulation of gel-based stimuli-responsive systems with tailored performances is the holy grail for many applications.

These considerations led us to explore the synthesis of innovative performing materials that combine specific properties of different moieties. For example we join the properties of vitamin C and sulphur, selenium or tellurium groups,[19] to the functional features imparted with the formation of supramolecular nanoassemblies. Along the same track, in this paper we report on the synthesis and physico-chemical characterization of 6-O-ascorbyl-oleate (Asc-OL), a bioconjugated surfactant obtained via enzymatic synthesis. Here the peculiar antioxidant properties of the two parent molecules combine with the hydrogen bonding and acidic features of the headgroups, the high fluidity of the hydrocarbon moiety, and the capacity to form self-assemblies in water with a large hydrophobic core.

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Results and Discussion

In this section the main results from DSC, SAXS, FTIR and radical activity experiments will be presented for Asc-OL (see Scheme 1) in the pure solid state or in aqueous dispersions as a function of the concentration.

Solid state.

Small-Angle X-ray Scattering. The SAXS data acquired on the pure solid lipid suggests the presence of a lamellar phase (see Figure S1 in the Supplementary Material). The profile shows the presence of three peaks at q = 0.154, 0.308 and 0.463 Å^{-1} due to the first, second and third reflections of the lamella. The corresponding spacing is d = 40.7 Å. The SAXS profile seems to exclude the coexistence of different polymorphs in the solid.

This result indicates that, if the hydrocarbon chains are oriented perpendicularly with respect to the plane formed by the headgroups, the interdigitation degree can be estimated at about 60%. Saturated single chained vitamin C surfactants (abbreviated as ASCn, where "n" refers to the number of carbons in the side chain) usually show a lower interdigitation degree.[20,21] In the present case the interdigitation is more extended. This is probably because of the large cross-section area of the heads that allows a deeper mutual interpenetration of the fluid oleoyl chains in the hydrophobic pocket.

Polymorphism. Polymorphism is a characteristic feature of fatty acids and similar molecules,[22] and refers to the ability of a molecule to take more than a single crystalline form, depending on its arrangement in the lattice: hexagonal (α_H), orthorhombic (β_O), triclinic (β_T), and monoclinic (β_M).[23] The formation of different polymorphs may depend on different experimental conditions during crystallization, such as choice of solvent, temperature, evaporation rate, etc.[24-26] Each polymorph is characterized by a specific spacing and the chain-length structure produces a periodic sequence of acyl chains in a unit cell lamella along the long-chain axis.[22] The polymorphic forms can be identified through WAXS and FTIR data.[27,28] WAXS profiles and FTIR spectra for the Asc-OL samples investigated in the solid state are shown in Figures S1-S3 in the Supporting Information. The four phases α_H, β_O, β_T and β_M produce specific bands between 750 and 710 cm^{-1} in the FTIR spectra (see Figures S2 and S3 in the Supporting Information).[20,29,30] According to our results Asc-OL forms an hexagonal phase in the solid state. We argue that the α_H phase – the less compact polymorph – is adopted by Asc-OL presumably because of the large cross-section of the polar heads of the presence of the liquid-like long unsaturated chains that keep the surfactant molecules from packing too tightly in the solid state.[31]

Differential Scanning Calorimetry. Pure Asc-OL melts at 62.4 °C with an enthalpy of fusion $\Delta_{fus}H$ of 15.3 kJ/mol, as detected from DSC. The entropy of fusion $\Delta_{fus}S = \Delta_{fus}H/T_{fus}$ is about 46 J/mol·K. Compared to saturated single chain ASCn, both the enthalpy and entropy of melting for Asc-OL are significantly lower. For example 6-O-ascorbyl-palmitate (ASC16) melts at 113.1° C with $\Delta_{fus}H$ = 53 kJ/mol and $\Delta_{fus}S$ = 137 J/mol·K.[32] We recall here that the large entropy of fusion of long chain saturated hydrocarbons are related to the onset at melting of rotational freedom of the molecule to change from one molecular configuration to another through rotations around C-C bonds.[33]

While the $\Delta_{fus}H$ of Asc-OL agrees quite well with the value indicated by Viklund *et al.* (between 8.8 and 13.2 kJ/mol),[34] the melting point is much lower than the temperature reported in the same study (83°-84° C). Correspondingly the value of $\Delta_{fus}S$ calculated from the data reported by Viklund is about 28 J/mol·K. In order to exclude water contamination, our Asc-OL sample was freeze-dried for 24 h at -55 °C and 30 mTorr. However, the repetition of the DSC scan in the same conditions indicated that the melting parameters did not change. In the work of Viklund and coworkers Asc-OL was purified by washing with hexane to remove unreacted oleic acid, and excess ascorbic acid was removed by first dissolving the solid in diethyl ether and then by filtration.[34] In our procedure, the synthesis product was treated in a different manner (see the Experimental section).

Furthermore the article by Viklund reports that Asc-OL exhibited only weak crystallinity, with two weak XRD peaks, a double chain packing with a spacing d = 35 Å, and a broad melting DSC peak at 83° - 84° C. The authors argued that the low value of enthalpy of fusion and the observed diffraction pattern indicate that Asc-OL solidifies primarily in a liquid crystalline structure with nonordered fatty acyl chains and a structured polar head group.[34] We conclude that presumably the less crystalline product isolated by Viklund *et al.* is a different polymorph compared to the Asc-OL we obtained.

Packing parameter. In order to predict the shape of the aggegates formed by Asc-OL in water we estimated the packing parameter p of the surfactant which is given by:[12,13]

$$p = \frac{v_H}{a_P l_H} \quad (1)$$

where a_P, v_H and l_H are the headgroup cross-section per molecule, the volume and the length of the hydrocarbon tail in its stretched conformation. In the case of Asc-OL, we used the density (0.895 g/mL) and molar mass (282.47 g/mol) of oleic acid to estimate v_H. Considering that long-chain carboxylic acids form dimers in the solid and liquid states, stabilized by two hydrogen bonds between the carboxylic groups,[32] the chain volume v_H is about 262 Å^3. The ChemBio3D Ultra software was used to estimate the length of the polar heads (l_{ASC}) and of the hydrophobic tails (l_H). They are about 8.0 and 20.4 Å, respectively. Finally, the value of a_P is estimated to range between 42 and 49 Å^2 from previous studies.[20] Combining these values, we obtain $p \approx 0.3$. This indicates that the surfactant is expected to form spherical particles dispersed in water.[12] We will use this information later when we discuss the SAXS results for the aqueous solutions of Asc-OL.

Aqueous dispersions.

Differential Scanning Calorimetry. Upon heating, the aqueous dispersions containing 5, 20 or 40% w/w of Asc-OL turn from white gel-like, viscous samples to transparent fluid liquids. The samples showed the presence of three peaks in DSC thermograms, encompassing the melting/freezing of bulk water at 0 °C, a micelle-to-coagel transition at about -5.8 °C in the cooling step, and a coagel-to-micelle phase transition at 13 °C during the heating step (see Figure 1). This is a typical behavior

in ascorbyl-based surfactants, in fact saturated analogues, *e.g.* ascorbyl palmitate, that bear a quite long alkyl chain, in water form stiff *coagels* (i.e. semicrystalline hydrated lamellae) that turn into gel-like transparent phases upon heating.[7,8,20,21,30,32,35-38]

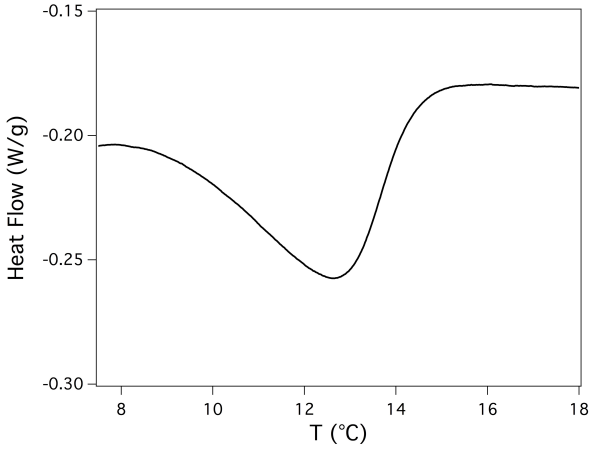


Figure 1. DSC scan of a 20% w/w aqueous dispersion of Asc-OL. Exo up.

The enthalpy changes increase almost linearly with the concentration of the lipid. From the experimental enthalpy change of fusion for bulk water (ΔH_{exp}, see Table 1) we calculated the amount of water that is strongly bound to the headgroups (W_b) as:[36]

$$W_b = (100 - P)\frac{(\Delta H_w - \Delta H_{exp})}{\Delta H_w} \quad (2)$$

here ΔH_w is the melting enthalpy for pure water and P (in % w/w) is the mass percentage of Asc-OL in the sample. Note that the enthalpy change measured by the instrument (ΔH_{instr}) is expressed in J/g of sample and needs to be converted into J/g of water through the formula:

$$\Delta H_{exp} = \frac{100 \cdot \Delta H_{instr}}{100 - P} \quad (3)$$

The number of strongly bound water molecules per single headgroup are calculated as:[36]

$$N_b = \frac{M_n \cdot W_b}{M_w \cdot P} \quad (4)$$

M_n and M_w are the molar masses of Asc-OL and of water, respectively.

The values of N_b are similar to those of other single chained ascorbyl surfactants, particularly when the lipid concentration is large.[36]

Small-Angle X-ray Scattering. SAXS experiments were carried out on aqueous dispersions of Asc-OL at 4° and 20 °C, i.e. below and above the transition temperature (see Figures 2 and 3).

The data were fitted with charged prolate core-shell micelles (for the structure and shape details see Figure 4).

Table 1. Experimental enthalpy change of fusion (ΔH_{exp}, in J/g of water), mass percentage (W_b, in % w/w) and number of strongly bound water molecules per single headgroup (N_b) at different surfactant concentration (P, in % w/w).

ASCn	P	ΔH_{exp}	W_b	N_b
Asc-OL[a]	4.43	329.6	5.1	28
	19.98	314.4	7.8	10
	34.25	305.6	8.1	6
ASC8[b]	4.87	308.9	7.1	24
	19.26	283.3	12.2	11
	37.46	254.0	15.0	7
ASC10[b]	5.03	306.9	7.6	28
	17.85	287.5	11.4	12
	37.91	255.4	14.6	7
ASC12[b]	4.85	320.9	3.7	15
	19.74	286.0	11.5	12
	39.17	251.5	15.0	8
ASC14[b]	4.51	320.7	3.7	18
	18.97	283.9	12.1	14
	40.25	250.0	15.0	8
ASC16[b]	5.41	315.3	5.2	23
	19.39	272.4	14.8	18
	42.02	239.2	16.4	9
BOLA12[c]	5.53	317.3	4.9	13
	18.98	287.8	8.9	9
	41.32	233.8	29.9	7

[a] this work; [b] from [36]; [c] from [37].

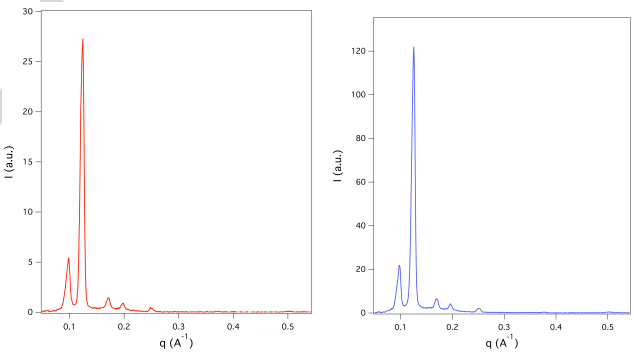


Figure 2. SAXS profiles for 20% (left) and 40% (right) aqueous dispersions of Asc-OL at 4 °C.

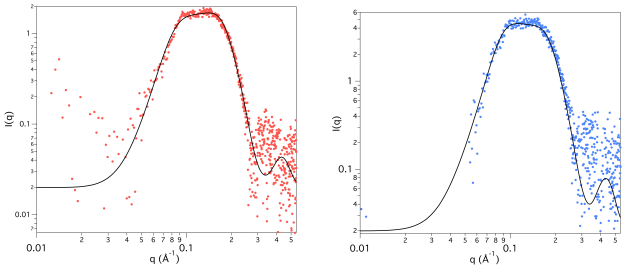


Figure 3. SAXS profiles for 20% (left) and 40% (right) aqueous dispersions of ASC-OL at 20 °C.

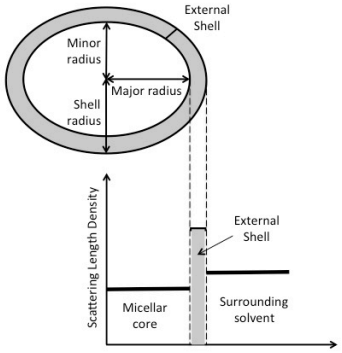


Figure 4. Structure of a micelle and contrast used in the fitting of the SAXS data.

In the SAXS experiment the scattering intensity *I(q)* is given by:

$$I(q) = KN_pV_p^2(\Delta\rho)^2P(q)S(q) + B \qquad (5)$$

here *K* is an instrumental constant, N_p the number density of the scattering particles, V_p the particle volume, $\Delta\rho$ the scattering length density contrast, *P(q)* the form factor, *S(q)* the structure factor and *B* the background term.

The expression for *P(q)* that appears in equation 5 is given by:

$$P(q) = \frac{scale}{V}\int_0^1 \left|F(q, r_{min}, r_{maj}, \alpha)\right|^2 d\alpha + bkg \qquad (6)$$

where

$$F(q, r_{min}, r_{maj}, \alpha) = V(r_{min}, r_{maj})(\Delta\rho)3j_1(u)/u \qquad (7)$$

$$u = q\left[r_{maj}^2\alpha^2 + r_{min}^2(1-\alpha^2)\right]^{1/2} \qquad (8)$$

$$V(r_{min}, r_{maj}) = (4\pi/3)\, r_{maj}r_{min}^2 \qquad (9)$$

$$j_1(x) = (\sin x - x\cos x)/x^2 \qquad (10)$$

The scale factor, the core and the shell radius, the scattering length density of the core, shell and solvent, and the background were the fitting parameters. The contrast $\Delta\rho$ is the difference in the scattering length density between the core and the shell. For the structure factor *S(q)* we adopted a model for charged ellipsoidal objects dispersed in a dielectric medium interacting via a screened Coulomb potential. Here the extracted structural fitting parameters are the core major and minor radii and the shell radius (see Figure 4). The scattering length density of the solvent was taken as that of pure water ($9.46\cdot10^{-6}$) while those of the shell and of the hydrophobic core were allowed to vary reaching values compatible with an ascorbic acid headgroup and an octadecene tail: about $9.6\cdot10^{-6}$ and $7.6\cdot10^{-6}$, respectively. The salt concentration obtained was compatible with the dissociation of the ascorbic acid moieties. The relative dielectric constant of the solvent is taken as that of pure water (80.36). The fitting parameters obtained are listed in Table 2.

Table 2. Structural fitting parameters extracted from the SAXS data of the 20% and 40% samples at 20 °C.

parameter	20%	40%
core major radius (Å)	19.1±0.5	20.3±0.5
core minor radius (Å)	12.3±0.4	12.1±0.4
shell radius (Å)	21.0±0.2	20.3±0.2
charge	2.8±1.5	3.6±1.2

The results are compatible with the dimensions of the Asc-OL molecule. In fact the core major and minor radii (19.1 and 20.3 Å, respectively) correspond to the length of a hydrophobic chain (20.4 Å) in a non-interdigitated lamellar structure. Substracting the core minor radius from the shell radius we obtain a size of about 8-9 Å, close to the value estimated for the length of a single ascorbyl moiety.[20] The results are in line with the calculation of the packing parameter *p* that corresponds to the formation of nearly spherical micelles.

Antioxidant activity.

In order to check the efficacy of ascorbyl-oleate against radical-induced oxidative degradation, we carried out some experiments to assess the protective activity performed by Asc-OL. In fact Vitamin C can inhibit radical-initiated lipid peroxidation, a process probably implicated in a variety of chronic health problems such as neurodegenerative, cancer and cardiovascular diseases.[38-41] However, due to its scarce solubility in lipophilic media Vitamin C is active only in aqueous environments.[20] Such drawback can be circumvented by transforming L-ascorbic acid into an amphiphilic molecule through the addition of one or more hydrophobic tails.

The reducing activity (RA, in %), evaluated with the DPPH method, was about 93.5%. The result confirms that Asc-OL keeps the same antioxidant properties of vitamin C.[38] Moreover, the comparison of the reducing activity of Asc-OL, L-ascorbic acid and other ascorbyl esters shows that these compounds possess at least the same antioxidant activity of the most common natural antioxidants (see Table 3).

The efficiency of Asc-OL in inhibiting lipid peroxidation in a lipophilic environment can be assessed by the ability to prevent or inhibit the oxidative degradation of ethyl linoleate in hexane induced by a lipophilic radical initiator, AIBN.[1,41] As shown in Figure 5, the absorbance at 234 nm increases, due to the

formation of a conjugated diene in the linoleic chain because of the radical attack. The addition of an ethanol solution of Asc-OL to the sample, indicated by the arrow in the plot, results in the end of the degrading action, and in a constant value of absorbance, even almost 10 hours after the addition of the ascorbyl derivative. The oxidation process will start again when the protection activity imparted by the ascorbyl derivative is over.

Table 3. Reducing Activity (RA, %) of Asc-OL compared to that of some other Vitamin C derivatives and of some natural antioxidants.[8,35,37,38]

compound	RA (%)
L-ascorbic acid	90.3
ASC8	89.3
ASC10	90.1
ASC12	90.3
ASC14	96.7
ASC18	96.1
Asc-OL	93.5
BOLA12	94.0
8ASC10	95.0
Di-ASCn	95.0
epicatechin	93.7
caffeic acid	94.5
rutin	88.7
oleuropein	89.8
tocopherol	86.8
melatonine	84.8

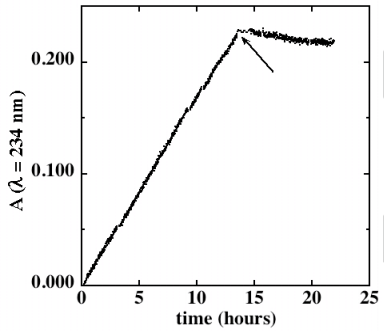


Figure 5. Evaluation of the inhibition of lipid peroxidation induced by the addition of Asc-OL to an ethanol solution of ethyl linoleate.

Conclusions

Ascorbyl-6-O-oleate (Asc-OL) was synthesized through an enzymatic reaction and was characterized in the pure state and in aqueous dispersion through DSC, FTIR and SAXS experiments. This molecule combines the redox and acidic properties of L-ascorbic acid to the liquid-like behavior of oleic acid. Asc-OL is a green amphiphile that self-assembles in aqueous dispersions due to the presence of a polar headgroups (Vitamin C) and of a long hydrophobic segment.

The results suggest the formation of small prolate, nearly spherical micelles in water dispersions, as predicted by the calculation of the packing parameter *p* of the lipid.

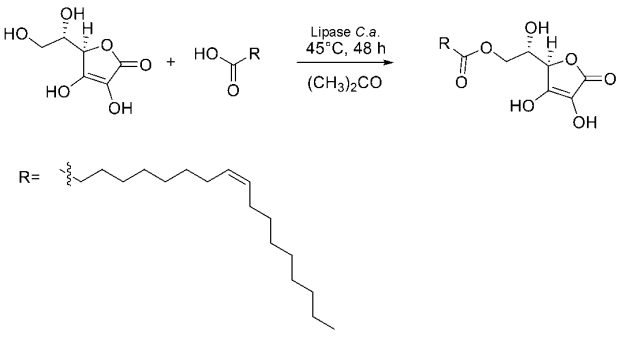
The formation of more concentrated hydrated lamellar structures (*coagels*) from Asc-OL will be the subject of future works in order to investigate their performance as drug delivery carriers, and their stimulus responsiveness to pH, ionic strength and nature of cosolutes, and addition of CO_2.

The radical scavenging tests, based on the DPPH method and on the control of the lipid peroxidation of ethyl linoleate, indicate that Asc-OL is a very promising candidate for protecting valuable and UV-sensitive products, both in the self-assembled state or in solution in the monomeric state. The presence of the liquid-like oleate moiety imparts a strong hydrophobic nature to the derivative, that favours its solubilization in lipophilic media and aggregation in functional nanostructures.

Experimental Section

L-ascorbic acid (> 99.0%), oleic acid (> 90%), Lipase immobilized from *Candida antarctica*, molecular sieves (4 Å, beads, 8-12 mesh), celite, ethyl linoleate and DPPH were purchased from Sigma-Aldrich (Milan, Italy). All reactants and solvents were used without further purification.

Synthesis. Ascorbyl-6-O-oleate (Asc-OL, MW = 440.575 g/mol) was obtained enzymatically by mixing L-ascorbic acid and oleic acid in acetone following the procedure summarized in Scheme 1.[42] 50 mmol of L-ascorbic acid were mixed with 10 mmol of oleic acid at room temperature in a capped vial. Lipase from *Candida antarctica* and activated molecular sieves (4 Å) were added under nitrogen atmosphere and the dispersion was magnetically stirred. Finally the proper amount of acetone was added through a Hamilton syringe. The vial was immersed in an oil bath and left at 45 °C for 48 h. After filtration of the raw product in ethyl acetate through celite, it was treated with water to remove the unreacted L-ascorbic acid and then extracted in ethyl acetate to recover Asc-OL. The organic layer was treated with Na_2SO_4, filtered and the solvent removed with a rotavapor. The crude solid was passed through a celite/Na_2SO_4 column and flushed with petroleum ether 40-60 °C. The collected final waxy solid was tested through ^{1}H- and ^{13}C-NMR (see Figures S4-S6 in the Supporting Information) and mass spectrometry.



Scheme 1. Enzymatic synthesis of Asc-OL in acetone.

^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm): 0.87 (3H, t, J=6.8 Hz, CH_3), 1.26-1.29 (20H, m), 1.53-1.67 (2H, m, CH_2CH_2CO), 1.98-2.05 (4H, m, $CH_2CH=CHCH_2$), 2.28-2.39 (2H, m, CH_2CO), 4.14-4.28 (2H, m, CH_aH_b e CHOH), 4.28-4.96 (1H, m, CH_aH_bO), 4.79 (1H, bs, CCHO), 5.29-5.37 (2H, m, CH=CH). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 14.1, 22.6, 24.8, 27.2, 27.2, 29.2, 29.2, 29.3, 29.3, 29.3, 29.5, 29.8, 31.9, 34.0, 64.0, 67.4, 76.2, 118.5, 129.6, 130.0, 153.5, 172.8, 174.1. MS (ESI, negative): 439 $(M-H)^-$. ATR-FTIR: 3300-3500 cm^{-1} (O-H stretching), 2800-2950 cm^{-1} (C-H stretching), 1770 cm^{-1} (C=O stretching), 1690 cm^{-1} (C=O and C=C stretching), 1400 cm^{-1} (CH_3 umbrella mode), 1100-1190 cm^{-1} (C-O stretching).

Sample preparation. The aqueous dispersions of Asc-OL were prepared by admixing the proper amounts of surfactant and Millipore water at 50 °C and then cooling down in a refrigerator at 4 °C. Three annealing cycles were performed to obtain a homogeneous sample.

Nuclear magnetic resonance. NMR spectra were acquired with a Varian Mercury 400 from Agilent Technologies Italia (Milan, Italy). Proton assignments were made according to spin systems, using COSY experiments.

Mass spectrometry. MS experiments were conducted on a Thermo-LCQ-Fleet (from Thermo Fisher Scientific, Waltham, MA, USA) using nitrogen as gas carrier and an ESI device.

Attenuated total reflectance Fourier-transform infrared spectroscopy. ATR-FTIR spectra were performed on a Nexus 970-FTIR (Thermo-Nicolet) from Thermo Electron Corp. (Waltham, MA, USA) with a resolution of 4 cm^{-1} and 128 scans, between 4000 and 600 cm^{-1}.

Differential scanning calorimetry. DSC scans were performed on a Q2000 TA instrument (Water SpA, Sesto San Giovanni, Italy) using aluminum pans sealed under inert atmosphere (nitrogen). The solid was first equilibrated for 5 mins at 0 °C and then heated up to 120 °C with a rate of 2 °C/min. For the aqueous dispersions the sample was kept at 0 °C for 30 mins, then cooled down to -90 °C and finally heated up to 60 °C.

Small angle X-ray scattering. SAXS measurements were carried out on a HECUS S3-MICRO camera (Kratky-type) equipped with a two position-sensitive detectors (OED 50M) containing 1024 channels of width 54 µm. CuKα radiation (wavelength l = 1.542 Å) was provided by an ultra-brilliant point micro-focus X-ray source (GENIX-Fox 3D, Xenocs, Grenoble), operating at a maximum power of 50W. The sample-to-detector distance was 281 mm. The volume between the sample and the detector was kept under vacuum during the measurements to minimize scattering from the air. The Kratky camera was calibrated in the small angle region using silver behenate (58.34 Å),[43] while lupolen (4.12 and 3.8 Å) was used as a reference for the wide-angle region. SAXS curves were obtained in the scattering vector (q) range between 0.01 $Å^{-1}$ and 0.54 $Å^{-1}$, where $q = (4p/l)sinq$, $2q$ being the scattering angle. The WAXS region covered in the experiment was from 1.3 $Å^{-1}$ to 1.9 $Å^{-1}$. Samples were filled into 1.5 mm glass capillaries. Solid samples were investigated at 10 °C, while gel samples were analyzed both below and above the transition temperature. For the latter, the temperature was set to 4 °C and 20 °C, respectively and controlled by a Peltier element, with an accuracy of ±0.1 °C. All scattering curves were corrected for the empty cell contribution considering the relative transmission factor.

Reducing activity and lipid peroxidation. The reducing activity (RA, %) was evaluated by measuring the absorbance at 517 nm of a DPPH (α,α-diphenyl-β-picrylhydrazyl) solution in ethanol (0.1 mM) before (A_0) and after (A_{20}) 20 minutes from the addition of an equal volume of the sample (0.1 mM in ethanol or water), as RA(%) = $100\cdot(A_0-A_{20})/A_0$. The lipid peroxidation study was performed through UV spectrophotometry on a 0.1 M solution of ethyl linoleate in *n*-hexane.[41] 2.5 mL of the solution were added to the cuvette (pure hexane as blank reference), and a few crystals of AIBN (recrystallized from methanol) were added in order to start the oxidation of the unsaturated chain. The reaction was monitored by measuring the absorbance at 234 nm, where the diene groups absorb. Finally, 0.1 mL of an ethanol solution of Asc-OL (10^{-4} M) were added to stop the lipid peroxidation (see the arrow in Figure 5, below). The data were corrected for dilution and the experiment was repeated three times.

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Keywords: L-ascorbyl esters • Vitamin C • oleic acid • antioxidant • micelle(s)

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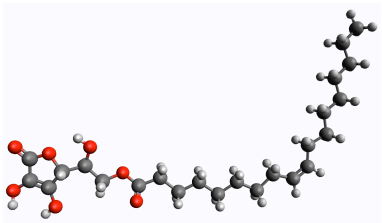

Ascorbyl-oleate (Asc-OL) is an antioxidant bioconjugate with an amphiphilic nature imparted by the presence of the hydrophilic Vitamin C head and the lipophilic, liquid-like oleic acid chain. New experiments elucidate the shape, size and thermal properties of the micellar aggregates produced in water. DPPH and linoleate tests suggest that Asc-OL is a green excellent candidate for the stabilization and vehiculation of valuable molecules.