Electronic Supplementary Information for:

"Pulling" π-conjugated polyene biomolecules into water: with the enhancement of light-thermal stability and bioactivity by a facile graphene oxide-based phase transfer approach

Wentao Zhang[a,b], Shaoxuan Yu[a], Wei Liu[a], Daohong Zhang[a], Wenxin Zhu[a], Yuhuan Zhang[a], Wanqiang Wu[a], Lixue Zhang[b*], Jianlong Wang[a*]

[a] College of Food Science and Engineering, Northwest A&F University, Yangling 712100, Shaanxi, China.
[b] Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, No. 189 Songling Road, 266101 Qingdao, China.

1. Materials and methods

1.1 Chemicals and Materials

Astaxanthin (purity > 95%) was purchased from Santa Cruz Biotechnology Co., Ltd.. Graphite powder (conducting grade, -325 mesh, 99.9995%) was purchased from Alfa Aesar China (Tianjin) Co., Ltd.. DPPH was obtained from Sigma-Aldrich Co. LLC (Shanghai, China). Hydrogen peroxide (H_2O_2) and sulfuric acid (H_2SO_4, 98%) were purchased from Xilong Chemical Co., Ltd.. Dimethyl sulfoxide (DMSO), dichloromethane, ethyl acetate, hydrochloric acid (HCl), sodium nitrate, potassium permanganate ($KMnO_4$) and ethanol were purchased from Shanghai Chemical Factory (Shanghai, China). CCK-8 was provided by Sangon Biotech (Shanghai) Co., Ltd.. The dialysis bag (MWCO = 8-14 kDa) was provided by Beijing Solarbio Science & Technology Co., Ltd.. Other chemicals were of analytical grade and used as received without further purification. Distilled water was used throughout all experiments.

1.2 Apparatus

The UV-vis spectra were measured with a UV-2550 spectrophotometer (Shimadzu, Japan) at room temperature. Transmission electron microscopy (TEM) measurements were performed with HT7700 (Hitachi, Japan) at 100 kV. Field emission scanning electron microscope (FESEM) image was taken by using an S-4800 (Hitachi, Japan). Fourier transform infrared (FT-IR) spectroscopy was recorded on a Vetex70 (BRUKER Corp., Germany). Microplate reader (Thermo Multiskan MK3) was also used to record OD values in CCK-8 assay.

1.3 Preparation of GO

All the glass ware used in the procedures was soaked and cleaned in a bath of freshly prepared aqua regia, rinsed thoroughly in pure water, and dried in air prior. Graphite oxide was synthesized from graphitic powder using a modified Hummer's method. [1,2] Briefly, 3 g graphitic powder and 2 g $NaNO_3$ were added to 70 mL of cold H_2SO_4 (98%) and left stirring acutely for 15 min. Afterwards, while keeping the temperature less than 20 ºC, 11 grams of $KMnO_4$ was added. This was stirred at less than 20 ºC for 30 minutes, and then stirred at 35 ºC, 50 ºC and 65 ºC for 20 minutes, respectively. Next, 138 mL 50 ºC distilled water was added, and the heat was increased to 95 ºC for 20 min. The reaction was ended by a final addition of 210 mL distilled water and 7 mL 30% H_2O_2 solution. For purification, the resulting substance was washed multiple times, first with 5% HCl solution and then with distilled water for 3 times each. Finally, the leftover was dried at 60 ºC overnight and collected. Before used, all of GO was sonication in water for 5 h, filtered though the 0.22 μm filter and than dialyzed against distilled water for two days to remove carryon impurities that could affect the properties of the material. [3]

1.4 Phase-transfer of AST by GO

Briefly, 10 mg astaxanthin was dissolved in 5 mL ethyl acetate and added to 10 mL of 1 mg/mL GO in water. The mixture was sealed under a nitrogen atmosphere and stirred at 25 ºC in the dark for 48 h. Then the solution was stood overnight for stratification and then the aqueous phase was collected. The produced GO-AST was preserved in darkness at 4 °C for further study.

1.5 AST quantitation in organic extracts of the complex

AST (1.0 mg) was dissolved into DMSO (10 mL), and then diluted to 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 μg/mL with DMSO, respectively. These samples were analyzed by a UV-vis spectroscopy, monitoring the absorbance from 300 to 600 nm. Then the regression equation was made between the peak absorbance at 491 nm (Y) and the concentrations (X) of AST. 2 mL of the GO-AST stock solution was freeze-dried and redissolved into 10 mL dichloromethane. The solution was subjected to an ultrasonic environment for 5 min and this was repeated three times. Then, the solution was centrifuged to collect the supernatant and the sediment was washed for another four times for further extraction. Finally, all supernatant was combined and dried, then redissolved into 40 mL DMSO and analyzed by UV-vis spectroscopy.

1.6 Storage stability

The solutions of AST in DMSO and GO-AST in water were prepared at 50 μg/mL. Each of samples were divided into three groups, put into transparent glass bottles and blanketed with N_2, then exposed to surroundings at 37 ºC and stored at 4 ºC and 37 ºC in dark. Then, the UV-vis spectrum of the sample was measured at least every 2 days.

1.7 DPPH radical scavenging activity

The DPPH radical scavenging activity assay was carried out by using a modified method. [4] Typically, 8 mg of DPPH was added into 100 mL of ethanol to receive 0.2 mM DPPH reagent. Then, 0.5 mL of this solution was added to 0.5 mL of sample solutions at different concentrations. The solution was allowed to react in dark for 30 min after mixing the antioxidants with the DPPH. The spectrum was scanned from 400 to 700 nm by the UV-2550 spectrophotometer. All of the tests were performed in triplicate, and the results were averaged. The DPPH radical scavenging rates (SR) were calculated by the following equation (1):

$$SR\ (\%) = [(A_0 - A_t) / A_0]*100 \qquad (1)$$

Where A_0 is the absorbance of 0.2 mM DPPH reagent, and A_t is absorbance of the supernatant after 30 min.

Besides, the DPPH radical scavenging activity of AST transferred by GO was calculated as:

$$SR_{AST\ in\ GO\text{-}AST} = SR_{total\ GO\text{-}AST} - SR_{GO} \qquad (2)$$

Where SR_{GO} and $SR_{total\ GO\text{-}AST}$ present the DPPH radical scavenging activity of GO and the total DPPH radical scavenging activity GO-AST, respectively, and $SR_{AST\ in\ GO\text{-}AST}$ is the DPPH radical scavenging activity of AST transferred by GO shown as in Fig. 4b.

1.8 Cell culture and Cell viability assay

HepG2 cell line, a human hepatocyte carcinoma cell line derived from a well-differentiated human hepatoblastoma, was provided from Collection of Cell Cultures of the Fourth Military Medical University (Shaanxi, China). HepG2 were cultured in RPMI-1640 medium supplemented with 10 % fetal bovine serum (FBS), benzylpenicillin (100 kU/L) and streptomycin (100 mg/L) at 37 ºC and in atmosphere of 5 % CO_2.

CCK-8 assay was used to assess the cell viability. HepG2 cells were seeded in 96-well plates in a density of 5000/well and incubated overnight. Cells were treated with different concentrations of GO, AST or GO-AST for 24 h, respectively. Following treatment, cells were rinsed with 1×PBS and treated with 10 μL CCK-8 reagent in serum-free media. After incubation for further two hours, the optical densities at 450 nm of each well in the plates were recorded using a Multiskan spectrum microplate reader and the results were expressed as a percentage of viable cells in comparison to the control (100 %).

1.9 Statistical analysis

All data were expressed as mean values ± standard deviation (SD). The intergroup variation was measured by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. The level of statistical significance was established at $p < 0.05$. All statistical analyses were performed using the Statistical Package for Social Sciences version 16 (SPSS Inc., Chicago, USA).

2. Results

2.1 The photos of phase-transfer and extraction of AST

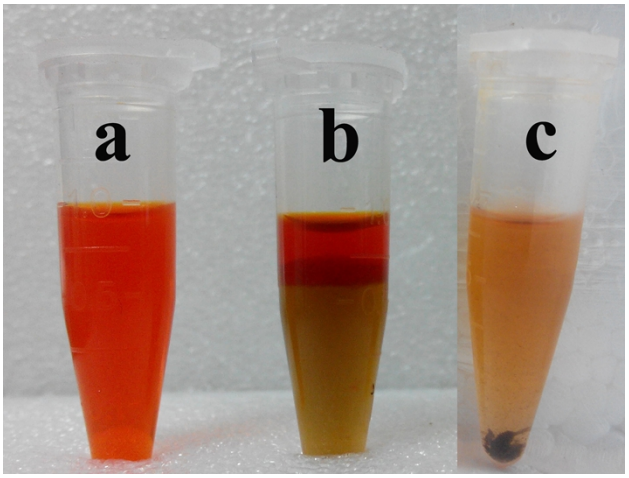

Fig. S1 The photo of AST in acetic ether (a), stratification of the mixing solution of AST in acetic ether (upper layer) and GO in water (lower layer) (b), and extraction of AST from freeze-dried GO-AST by dichloromethane (c).

The mixing solution of AST in acetic ether and GO in water was stood overnight for stratification and the photo was shown in Fig. S1b. The mixing solution was distinctly divided into two layers, and the upper layer was AST in acetic ether and the lower layer was water contained GO (might load some AST molecules). The lower layer was then collected, dialyzed and freeze-dried. Subsequently, the freeze-dried GO-AST was redissolved in dichloromethane and the photo was displayed in Fig. S1c. Obviously, dichloromethane solution turned red, while GO did not dissolve in dichloromethane, which indicates that AST can be extracted from GO-AST by dichloromethane.

2.2 The structure of AST and AST with intramolecular hydrogen bonds

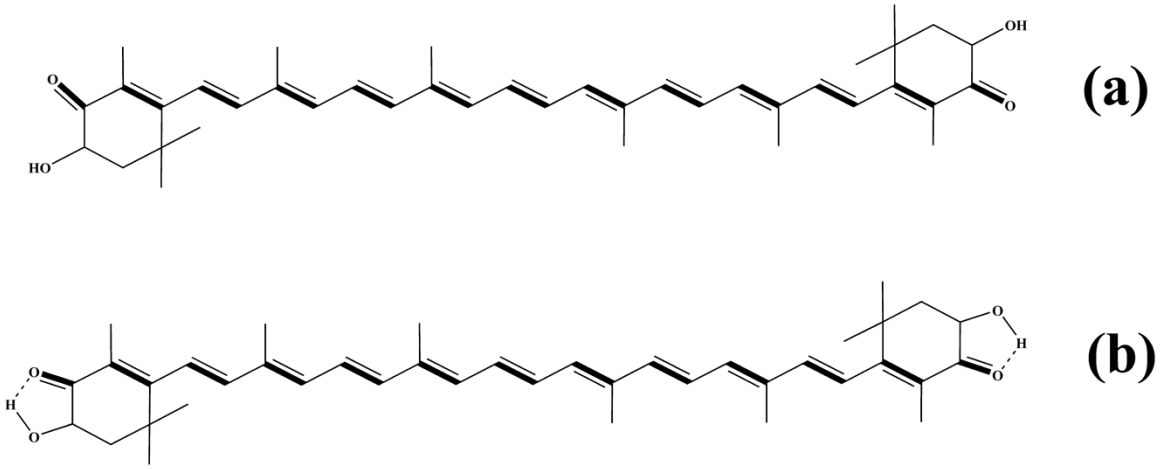

Fig. S2 Structure of (a) AST and (b) AST with intramolecular hydrogen bonds (The dashed lines show hydrogen bonds.).

2.3 DPPH scavenging activity of GO

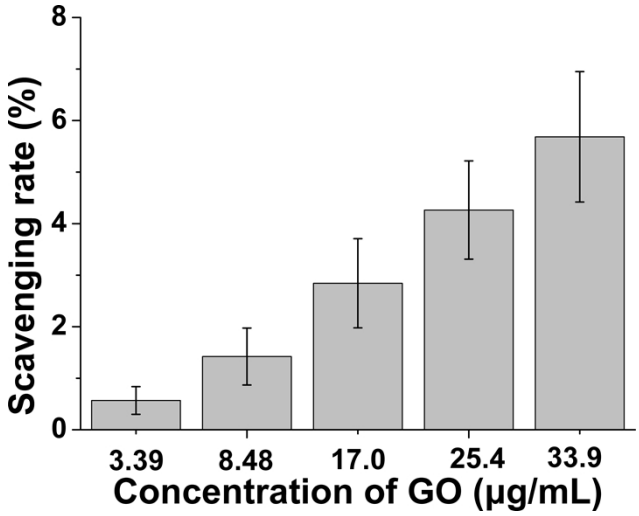

Fig. S3 DPPH scavenging activity of GO with various concentrations.

2.4 The release properties of AST from GO

The release properties of AST from GO were studied in water and PBS buffer. However, no AST was released from GO when water or PBS buffer was used as extraction reagent because of both the poor solubiliy of AST in water or PBS and the strong π-π stacking interaction of AST with GO. Those results indicate AST in GO-AST complex exerts its chemical and biological activities through the direct contact of GO-AST complex with DPPH free radicals or cells, rather than the release of AST from GO-AST complex.

References

1. Z. Liu, J. T. Robinson, X. Sun and H. Dai, *J. Am. Chem. Soc.*, 2008, **130**, 10876-10877.
2. D. C. Marcano, D. V. Kosynkin, J. M. Berlin, A. Sinitskii, Z. Sun, A. Slesarev, L. B. Alemany, W. Lu and J. M. Tour, *Acs Nano*, 2010, **4**, 4806-4814.
3. O. N. Ruiz, K. A. S. Fernando, B. Wang, N. A. Brown, P. G. Luo, N. D. McNamara, M. Vangsness, Y.-P. Sun and C. E. Bunker, *Acs Nano*, 2011, **5**, 8100-8107.
4 Y. Okada and M. Okada, *J. Agric. Food. Chem.*, 1998, **46**, 401-406.