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Interaction of Sodium Dodecyl Sulfate with Watermelon Chromoplasts and Examination of the Organization of Lycopene within the Chromoplasts

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The properties of plant-derived precipitates of watermelon lycopene were examined in aqueous sodium dodecyl sulfate (SDS) as part of an ongoing effort to develop simpler, more economical ways to quantify carotenoids in melon fruit. Levels of SDS >0.2% were found to increase the water solubility of lycopene in the state in which it was isolated from watermelon. Electron microscopy and chemical analyses suggested that the watermelon lycopene as isolated is packaged inside a membrane to form a chromoplast. Spectral peaks in the visible region of the watermelon chromoplasts in SDS exhibited a bathochromic shift from those in organic solvent. Watermelon chromoplasts in SDS exhibited pronounced circular dichroic activity in the visible region. Binding measurements indicated that about 120 molecules of SDS were bound per molecule of lycopene inside the chromoplast; likely, the detergent molecules are bound to the chromoplast membrane. Around 80% of the chromoplast−SDS complexes were retained on a 0.45 μm membrane filter. Together, these observations are consistent with lycopene in a J-type chiral arrangement inside a membrane to form a chromoplast. The binding of SDS molecules to the chromoplast membrane form a complex that is extensively more water-soluble than the chromoplast alone.

KEYWORDS: Lycopene; watermelon; carotenoids; detergents; SDS; chromoplasts

INTRODUCTION

A substantial number of studies have demonstrated an inverse relationship between the consumption of certain fruits and vegetables and the risk of cancer (*1*). This protective effect has been generally ascribed to the carotenoids contained in those foods (*2*). In the contemporary food industry, food supplements have become more and more prominent as a result of the increased production of prepared, processed, and convenience foods. Carotenoids are one of the classes of compounds occupying an ever-increasing role as food supplements. Nutritional and physiological effects of carotenoids are, in general, limited by their bioavailability. Increased bioavailability of carotenoids can be achieved by forming nanoparticles of the carotenoid in a hydrophilic matrix (*3*) or in a microemulsion (*4*). Considerable effort has been made to delineate the aggregated state of carotenoids as they exist in various size ranges of nanoparticles (*3*, *5*, *6*, *7*).

Lycopene, a fat soluble carotenoid, is a precursor of β-carotene (*8*) and has at least twice the antioxidant capacity of β-carotene (*9*). A number of epidemiological studies suggest positive health benefits to be derived from the consumption of diets high in lycopene (*10*), although a consensus for either its beneficial or detrimental role in the modulation of carcinogenesis remains to be established (reviewed in refs *11* and *12*).

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As part of an ongoing effort to develop simpler, more economical, and more environmentally friendly ways to quantify lycopene in watermelon tissue, the behavior of watermelon tissue exposed to aqueous solutions of a number of different types of detergents was examined. Particularly interesting were the alkyl sulfates, notably sodium dodecyl sulfate, SDS. The behavior of plant-derived precipitates of watermelon lycopene in SDS is described herein.

MATERIALS AND METHODS

Preparation of Watermelon Lycopene Chromoplasts. A total of 14 cultivars of watermelon (*Citrulles lanatus* (*Thumb.*) Mansf.) were employed in the study. They represented seedless, hybrid seeded, and open-pollenated seeded types of watermelon. Heart tissue from a ripe watermelon was homogenized to a smooth puree in a blender (Waring Products, New Hartford, CT). The puree was squeezed in a silk parachute cloth to separate the juice from the pulp. The nominal dimensions of the largest pores in the cloth were 35 μm × 70 μm. The juice, which we observed to contain ~50% of the total watermelon lycopene, was centrifuged at 11 700*g* for 30 min at 20 °C to pellet the lycopene. The lycopene pellet was washed twice with deionized H_2O by resuspending the pellet to one-half of its original volume and centrifuging to remove the water wash. Deionized water was then added to the washed pellet to make a slurry of nominal lycopene with a concentration of ~6 mg/mL. Its carotenoid composition by HPLC analysis (*13*) was nominally 90% *trans*-lycopene, 7% cis-isomers of lycopene, and 3% β-carotene.

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Direct Solubilization of Carotenoid-Containing Bodies from Watermelon with SDS. To 2 g of watermelon tissue puree, prepared as described previously, was added 6 mL of 0.4% SDS in H_2O. Sodium azide, NaN_3, can be included in the solution at a level of 0.02% to prevent bacterial and mold growth if the SDS solution is to be stored at room temperature for long periods of time. For acidic fruit such as grapefruit, it was necessary to adjust the pH of the puree to near neutrality before the SDS could effectively solubilize the chromoplasts. The suspension was ground in a 10 mL Potter−Elvenjheim homogenizer at 200 rpm to ensure complete disruption of the tissue. After standing for 1 h to allow denaturation/solubilization of cellular components, the homogenate was centrifuged at room temperature for 15 min in a 15 mL conical centrifuge tube at 1500*g* (3000 rpm) in a Sorvall TC6 bench top centrifuge (Kendro Laboratory Products, Asheville, NC). The carotenoid-containing particles were found suspended in the aqueous supernatant, and the colorless tissue residue was pelleted at the bottom of the tube. On rare occasions, some of the residue in a sample would float rather than pellet. By resuspending the sample, briefly degassing it with house vacuum, and recentrifuging it, all of the solid material would pellet.

Quantitative Assays. Lycopene was routinely quantified by the low volume hexane extraction method (*14*). In selected instances, the distribution of lycopene geometric isomers was determined by HPLC (*13*). SDS was quantified by the method of Mukerjee (*15*) as modified by Hayashi (*16*). The critical micelle concentration (CMC) of SDS under various solution conditions was estimated by the dye solubilization method (*17*, *18*) with the use of the dye, Sudan II (2,4-dimethyl-1-phenylazo-2-napthol; CAS# 3118-97-6; C.I. #12140) (Aldrich). The CMC of SDS in deionized H_2O estimated using this dye, 0.22%, agreed with literature values (*17*).

Physical and Chemical Measurements. Transmission electron microscopy of lycopene-containing microbodies from red watermelon flesh was performed by the Microscopy and Imaging Center, Texas A&M University, College Station, TX. Specimens were fixed in 2.5% buffered glutaraldehyde, washed in the same buffer, and then post-fixed in buffered 1% osmium tetroxide. Specimens were dehydrated in a graded ethanol series and infiltrated and embedded in epoxy resin. Thin sections on grids were post-stained with 2% uranyl acetate followed by Reynolds lead citrate (*19*). Grids were examined and photographed in a JEOL 1200EX transmission electron microscope at an accelerating voltage of 100 kV.

A watermelon chromoplast preparation was examined for the presence of membrane-associated lipids by solvent extraction and thin layer chromatography. The preparation, as an aqueous paste, was extracted twice with successive 10× volumes of chloroform−methanol (2/1: v/v). The two extracts were combined and evaporated to near dryness under N_2. The residue was dissolved in 2 mL of chloroform, treated with anhydrous sodium sulfate, and evaporated to a volume of ∼0.3 mL. Aliquots of the extract were chromatographed on Whatman PK5 silica gel TLC plates that were developed in chloroform/methanol/water (65:25:4: v/v/v) (*20*). Commercial standards (Sigma) of phosphatidyl inositol, phosphatidyl ethanolamine, phosphatidyl choline, and lyso-phosphatidyl choline were run with the unknown. Resolved components were visualized with I_2 vapors. Material remaining after lipid extraction was solubilized by heating in 2% sodium dodecyl sulfate (SDS) under reducing conditions. The proteins from this solubilized extract were subjected to SDS gel electrophoresis (*21*).

Absorption spectra and absorbances were measured with a Perkin-Elmer 3B spectrophotometer whose absorbance accuracy was checked with standard dichromate (*22*). Absorption scans of lycopene chromoplasts in SDS showed pronounced light scattering. Data were collected between 850 and 700 nm where the carotenoids appeared to exhibit no intrinsic absorbance, and these data were used to estimate a light scattering correction. The corrections employed utilized an inverse power term of the wavelength (*23*). The precision of the determination of the power to which the ratio of wavelengths was raised was about ±15%. Ten individual preparations of watermelon chromoplasts went into estimating these numbers, while over 100 samples were used to estimate the value for direct SDS extracts of watermelon tissue. Thus, to correct the visible absorbance values for light scattering effects in SDS for each preparation, the following equations were employed:

direct extract of watermelon tissue:

$$A_{\lambda,\text{ls corr}} = A_{\lambda,\text{obs}} - (A_{700})(700/\lambda)^{3.0}$$

fresh or frozen chromoplasts: $A_{\lambda,\text{ls corr}} = A_{\lambda,\text{obs}} - (A_{700})(700/\lambda)^{2.5}$

Circular dichroism spectra in the visible region were measured in the laboratory of K. Rodgers, University of Oklahoma Health Sciences Center, with a Jasco model J-715 instrument (Jasco Ltd., Japan). The concentration of lycopene in the samples employed for the CD measurements was 5.81×10^{-5} M (31.2 μg/mL) in 0.2% SDS. Measurements were performed in a 1 cm path length quartz cell.

Solubility of the watermelon chromoplasts in aqueous SDS was determined as follows. The chromoplast preparation was suspended in 0.02% sodium azide in water to a level of ∼2 mg/mL. To a 15 mL conical centrifuge tube with a screw cap were added: 2.0 mL of the chromoplast suspension, a volume of 1% SDS in 0.02% sodium azide to give the desired final concentration of SDS, and a volume of 0.02% sodium azide in water to give a total suspension volume of 10.0 mL. Samples were mixed well on a vortex mixer and then placed on a rotating shaker at 100 rpm and shaken for 24 h at 22 °C. Samples were then centrifuged at 11 700*g* for 1 h at 22 °C on a Sorvall RC5B centrifuge (Kendro Laboratory Products, Asheville, NC). Supernatant was removed and centrifuged again. Aliquots of the supernatants were assayed for lycopene by the low volume hexane extraction method (*14*).

Gel chromatography was performed in a 1.6 cm × 95 cm column (Amersham Pharmacia Biotech, Piscataway, NJ) of Sepharose CL-6B (Sigma Chemical, St. Louis, MO) equilibrated in 0.2% SDS + 0.02% NaN_3 in H_2O and operated at room temperature. A flow rate of 2 mL/h/cm^2 was maintained with a peristaltic pump (Amersham Pharmacia Biotech, Piscataway, NJ). Fractions of ∼1.3 mL were collected. Selected fractions were monitored for lycopene by absorbance at 565 nm.

The binding of SDS to a watermelon chromoplast preparation was quantified by equilibrium dialysis and subsequent assays for detergent and lycopene. Chromoplasts suspended in 0.2% SDS + 0.02% sodium azide in water were placed in dialysis bags, and deionized water was placed in separate bags. Samples were dialyzed versus 0.2% SDS plus 0.02% sodium azide at room temperature (22 °C) in an amber container that was additionally covered with a wrap of aluminum foil. The material inside those dialysis bags that started out containing only deionized water was sampled periodically until the SDS concentration inside those bags equaled the SDS concentration outside the bags. This process reached equilibrium within 10 days and was used as an indication that the system had reached dialysis equilibrium. During this period, no loss of chromoplasts from the dialysis bags was detected (as indicated by lycopene levels). Once the system reached dialysis equilibrium, the contents of those dialysis bags containing the chromoplasts and the dialysis medium outside the bags were assayed for SDS and lycopene.

RESULTS

A variety of amphiphiles was evaluated for their ability to solubilize lycopene from watermelon tissue. **Table 1** summarizes these results. Detergents were evaluated either at or just below their reported CMC in water and at a concentration sufficiently greater than the CMC to ensure a significant concentration of detergent micelles. Of the detergents that were tested, only the *n*-alkyl sulfate detergents, *n*-octyl sulfate, and *n*-dodecylsulfate (SDS) appeared to solubilize appreciable quantities of lycopene. The carotenoids solubilized in the *n*-alkyl sulfates were subsequently extracted with hexane, and the total amount of extracted lycopene was compared to that directly extracted from identical tissue by hexane. The results indicated that the *n*-alkyl sulfate solutions had extracted >95% of the lycopene from the watermelon tissue. That the carotenoids in watermelon tissue had indeed been extracted with the *n*-alkyl sulfates was also confirmed by reversed phase HPLC (*13*).

Table 1. Extractability of Watermelon Lycopene by Various Amphiphiles[a]

amphiphile	concentration (%)	A_{565}	$A_{565,\ corr}$ [b]
sodium octyl sulfate	3.0	0.89	0.41
sodium octyl sulfate	5.2	0.87	0.43
sodium dodecyl sulfate	0.2	0.88	0.44
sodium dodecyl sulfate	0.4	0.89	0.45
CHAPS	0.4	0.24	0.19
CHAPS	0.7	0.14	0.07
octyl glucoside	0.7	0.34	0.23
octyl glucoside	0.93	0.22	0.12
Triton X-100	0.02	0.62	0.01[c]
Triton X-100	0.22	0.39	0.24
CTAB	0.04	0.12	0.004
CTAB	0.24	0.05	0.005
sodium stearoyl lactate	0.4		0.002
unsaturated monoglyceride	0.4		0[c]
sucrose palmitate	0.4		0.001[c]
sucrose stearate	0.4		0.02
sucrose laurate	0.4		0.06
Tween 80	0.4		0.02[c]

[a] Each detergent was evaluated at a concentration below its CMC in water and at a second concentration well above its CMC. Each sample was centrifuged to remove cellular debris, and its apparent absorbance was determined at 565 nm. [b] Light scattering correction used: $A_{565,\ corr} = A_{565} - A_{700}(700/565)^3$. [c] Extract milky; little or no red material extracted.

Because of cost, availability, and relative quantities of required detergent, additional experiments were conducted with SDS only. The level and state of aggregation of SDS necessary to extract lycopene from watermelon tissue was investigated by treating 2 g samples of watermelon tissue with 6 mL of SDS of varying concentrations. At the same time, the cmc of SDS was determined in a solution of three volumes of water plus one volume of carotenoid-free watermelon juice. Under the conditions of this experiment, extraction of lycopene increased from 0 to 0.05% SDS and remained constant at all SDS levels between 0.05 and 0.5%. The cmc of SDS under these conditions as estimated by dye solubilization was 0.05%. HPLC analysis of lycopene extracted from watermelon tissue with aqueous 0.3% SDS exhibited the same levels, statistically, of all-*trans*-lycopene and its cis-isomers as those of direct hexane extracts of the tissue. Using 0.3% SDS, the carotenoids could be extracted from watermelon, pink grapefruit, guava, cantaloupe, fresh red tomatoes, and papaya. For acidic fruit such as grapefruit, it was necessary to adjust the pH of the puree to near neutrality before the SDS would completely extract the carotenoids. Conversely, only limited quantities of carotenoids could be extracted from processed tomato products, even after pH adjustment. From the processed tomato sources, only small amounts of lycopene were extracted, along with larger quantities of α- and β-carotenes. Attempts to improve lycopene extraction from tomato sauce by heating at 60 °C for up to 30 min or at 100 °C up to 15 min did not appreciably increase the amounts of carotenoids solubilized. No significant difference in the amount of lycopene extracted from watermelon tissues (or loss after solubilization) could be detected for extra treatment times with 0.2% SDS at room temperature from 1 h up to 24 h or at 60 °C from 10 min up to 30 min. Samples treated at 100 °C for longer than 5 min began to show a decline in lycopene levels. It was also observed that only part of the lycopene from noticeably over-ripe watermelons could be extracted into 0.2% SDS. Centrifugation after SDS treatment of decidedly over-ripe watermelon yielded a band of intense red material pelleted on top of the colorless cell debris.

Lycopene-containing material was prepared from watermelon in two different ways. One batch was prepared in the juice of the watermelon, and one batch was prepared by extraction from watermelon flesh into 0.3% SDS. **Figure 1** shows transmission electron micrographs of the two samples. Under both sets of conditions, spherical microbodies can be seen that are similar to the lycopene-containing chromoplasts in tomatoes (*24*). Diameters of the watermelon chromoplasts ranged from 0.1 μm to as large as 6 μm. Also observed in the electron micrographs were amorphous materials that likely represent residual cell debris. Thus, it appeared that the SDS was solubilizing the lycopene-containing chromoplasts as a whole rather than disrupting the chromoplast membrane and solubilizing the lycopene itself.

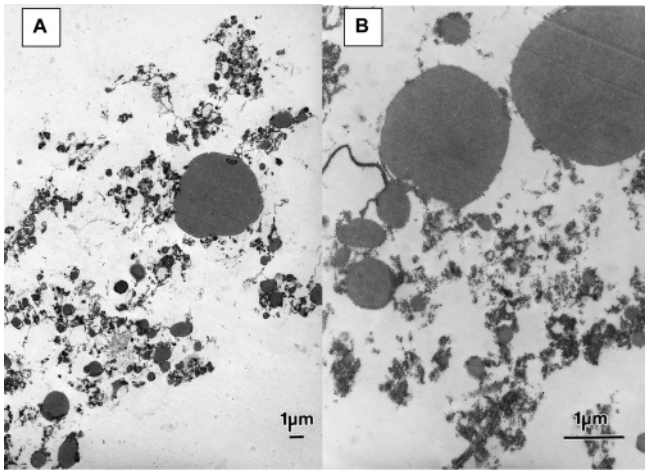

Figure 1. Transmission electron micrographs of watermelon juice and an SDS extract of watermelon fruit flesh. Samples were prepared as described in the Materials and Methods. (**A**) Watermelon juice at a magnification of 3750×. (**B**) Watermelon flesh extracted with 0.3% SDS at a magnification of 15000×.

In an attempt to better define the chemical nature of the chromoplast membranes surrounding the watermelon lycopene, chromoplast preparations were extracted with chloroform−methanol, and the extract was dried under helium, redissolved in chloroform, and subjected to thin layer chromatography. Lycopene and β-carotene ran near the solvent front. Substantial amounts of compounds were observed that migrated commensurate with phosphatidyl inositol, phosphatidyl ethanol, and phosphatidyl choline. Several unidentified compounds of lesser quantities were also observed. Material remaining after lipid extraction was solubilized in SDS and subjected to SDS gel electrophoresis. Predominant protein bands of molecular weights of 52, 40, 31.2, 24.3, 23.5, 14, and ~10 kDa were observed. Thus, the phospholipid and protein data are consistent with membrane-surrounded structures for the nature of the lycopene-containing bodies obtained from watermelon.

Physical State of Carotenoid-Containing Bodies in Aqueous SDS. In an attempt to better understand the ability of SDS to solubilize carotenoids still encapsulated in a membrane, selected properties of the carotenoid complex in aqueous SDS were investigated. **Figure 2** summarizes the apparent solubility of lycopene-containing watermelon chromoplasts in aqueous SDS solution. As illustrated by the figure, watermelon chromoplasts are soluble in aqueous SDS to a level of ~185 μg of lycopene/mL. As described previously, watermelon chromoplasts in SDS are very large. Thus, the apparent solubility of the carotenoid preparation in SDS is a reflection of the centrifugal force to which the saturated solution is exposed before measuring the concentration of carotenoid left in the supernatant. Centrifuging the suspension at 2000*g* relative

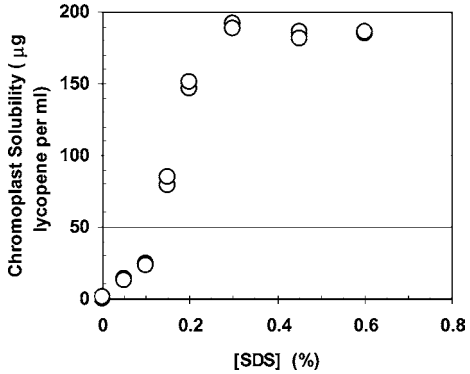

Figure 2. Solubilities of watermelon chromoplasts in 0.2% sodium dodecyl sulfate. Measurements were performed on chromoplasts equilibrated in 0.2% SDS + 0.02% sodium azide at 22 °C. The experimental points represent chromoplast–detergent complexes that remained suspended after centrifugation at 11 700*g* relative centrifugal force for 1 h. Lycopene levels were used as a measure of chromoplast levels. Lower centrifugal force fields yielded a higher apparent solubility (see text).

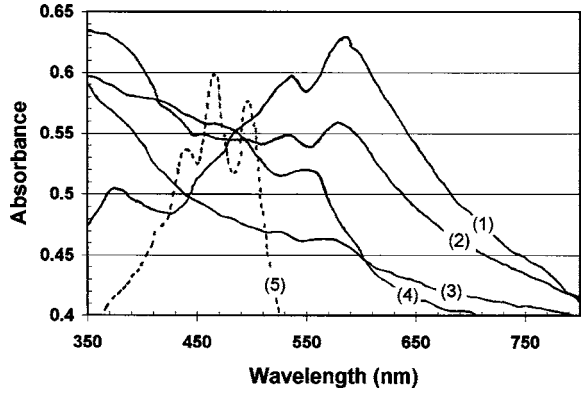

Figure 3. Spectral properties in the visible region of lycopene from various sources. Spectra were measured at 23 °C in a 1.0 cm quartz cuvette. Samples were centrifuged for 15 min at ~100*g* before spectral measurements were taken. Corrections for light scattering were not applied to the absorbance data. Curve 1: commercial lycopene suspended in 0.5% SDS for 10 h at room temperature before spectral measurement. Curve 2: commercial lycopene suspended in 0.5% SDS for 5 days at room temperature before spectral measurement. Curve 3: commercial lycopene suspended in 0.5% SDS for 12 days at room temperature before spectral measurement. Curve 4: watermelon chromoplasts extracted directly from watermelon tissue into 0.2% SDS. Curve 5: lycopene, either commercial or from watermelon chromoplasts, dissolved in *n*-hexane.

centrifugal force rather than at 11 800*g* left a concentration of ~450 μg/mL lycopene in the supernatant at saturation.

The interaction between watermelon chromoplasts and SDS in an aqueous environment was also examined by equilibrium dialysis. By this method, SDS bound to the chromoplast membranes at a level of 120 molecules of SDS bound to the membrane per molecule of lycopene inside the membrane. HPLC analysis indicated that no changes in carotenoid composition or isomer distribution had occurred during the 10 days of dialysis at room temperature that it took to reach system equilibrium.

Figure 3, spectrum 4 represents the absorption spectrum of lycopene in 0.2% SDS as it occurs in the watermelon chromoplasts. The spectral maxima were distinctly red-shifted from their respective positions in hexane (**Figure 3**, spectrum 5) or other organic solvents (*25*, *26*). The peak shift is about 60 nm. Apparent peaks were observed at 565 and 525 nm. Watermelon chromoplasts containing lycopene yielded the same spectrum whether fresh or frozen before solubilizing in aqueous SDS. By centrifuging a chromoplast preparation in water at lower speeds (~2000*g*), it was possible to obtain a chromoplast suspension of sufficient concentration to obtain an absorption spectrum of the packaged carotenoid in an aqueous environment absent of detergent (curve not shown). Qualitatively, the peaks at 565 and 525 nm that were seen for chromoplasts in SDS were also seen for them in water alone. The apparent molar absorptivity of lycopene appeared to be ~15−20% smaller in water than in 0.2% SDS, although this may be the result of additional particulate-enhanced spectral flattening (*27*). The absorbance at 565 nm of lycopene-containing chromoplasts at various concentrations (as measured by lycopene concentration) in aqueous SDS obeyed Beer's law. This was true whether the absorbance was corrected for light scattering or not. The absorptivity value at 565 nm in 0.3% SDS based on lycopene concentration and corrected for light scattering was 10 147 ± 1070 M^{-1} (0.0189 ± 0.002 mL/μg), and that not corrected for light scattering was 17 717 ± 1611 M^{-1} (0.033 ± 0.003 mL/μg). The lycopene value estimated for each of the 180 watermelons by extraction and absorption in SDS was compared with its corresponding estimate by hexane extraction. Lycopene values from the SDS extraction showed a direct one-to-one linear correlation to the values by hexane extraction, both with a light scattering correction (slope = 0.946; r^2 = 0.91) and without a light scattering correction (slope = 0.979; r^2 = 0.90).

We felt it to be of interest to examine the behavior of a commercial sample of lycopene that had been prepared by solvent extraction and thus was no longer packaged inside a membrane and for which the lycopene had been monodispersed while in an organic solvent. A dried commercial preparation of tomato lycopene (Sigma) was suspended in 0.5% aqueous SDS at a level of 55 μg/mL by repeated sonication and stirring over a 10 h period. The sample then sat in the dark at room temperature over a 2 week period, and its spectrum was measured periodically over this time. **Figure 3**, spectra 1−3 illustrate the spectral changes that occurred in the sample over time. As can be seen from the spectra, the lycopene suspension initially exhibited peaks at 588, 534, and 377 nm. As time progressed, the two peaks at longer wavelengths shifted to shorter and shorter wavelengths and approached that observed for watermelon chromoplasts. At 5 days, the peaks were at 578 and 533 nm. At 12 days, the peaks were at 569 and 523 nm. Additionally, the size of the lycopene aggregate apparently changed as evidenced by the increasing apparent absorbance (i.e., light scattering) at shorter wavelengths.

In an effort to gain further insight into the organizational nature of lycopene as it exists in the chromoplasts, the circular dichroism spectrum of a watermelon chromoplast preparation solubilized in 0.2% SDS was measured in the visible region. The CD spectrum between 300 and 700 nm is shown in **Figure 4** together with the visible absorption spectrum corrected for light scattering. A rather interesting CD spectrum is observed. Positive ellipticity bands are observed near the absorption maxima at 565 and 525 nm. A broad, asymmetrical positive ellipticity peak occurs at 380 nm. The sense of the ellipticity changes from positive to negative around the region of maximal absorbance at 346 nm. Weak negative double ellipticity bands are seen near 600 and 330 nm, although the signal-to-noise ratio around 330 nm reduces the confidence for an unambiguous identification of a double negative trough as opposed to a broader, single trough.

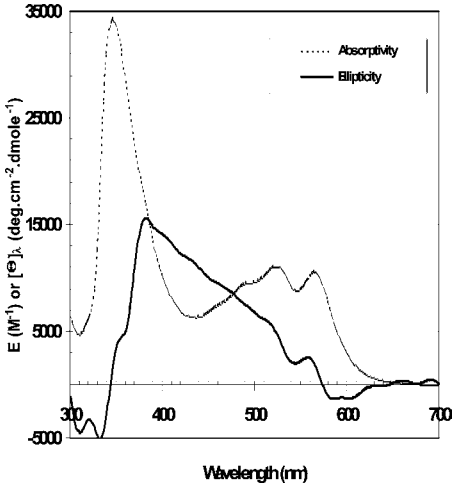

Figure 4. Spectral properties in the visible region of watermelon chromoplasts in 0.2% sodium dodecyl sulfate. The CD spectrum (solid line) was measured at 25 °C in 0.2% SDS at a lycopene concentration of 58.1 μM in a 1.0 cm path length quartz cuvette. The absorption spectrum (dashed line) of lycopene was measured at 23 °C in a 1 cm path length quartz cuvette. Lycopene concentration for the scan between 400 and 700 nm was 58.1 μM and for the scan between 300 and 400 nm was 29.1 μM. Corrections for light scattering as outlined in the text were applied to the absorption data.

To obtain a further estimate of the size of the watermelon chromoplasts, they were subjected to gel chromatography on 6% agarose equilibrated in 0.2% SDS. The lycopene-containing chromoplasts eluted in the column void volume. The pore distribution of 6% agarose thus suggests that the effective hydrodynamic radii of the chromoplasts were greater than 13 nm. When subsequently tested, ~87% of the lycopene was observed to be retained when the chromoplast preparation suspended in 0.2% SDS was filtered on a 0.45 μm filter.

DISCUSSION

Plant-derived precipitates of watermelon lycopene appear by electron microscopy to consist of lycopene packaged inside a phospholipid membrane to form a chromoplast or thylakoid similar to those observed in red tomato (*24*). Furthermore, these preparations of chromoplasts contain significant levels of phospholipids and a number of proteins that are solubilized only under denaturing conditions. These results are consistent with a surrounding membrane associated with the lycopene. The presence of a membrane enclosing the hydrophobic lycopene renders the watermelon chromoplast water-insoluble. The binding by an anionic detergent such as SDS to the chromoplast membrane increases the water solubility of the chromoplasts. Normalized to their lycopene content, the level of anionic detergent that binds to the membrane is equivalent to 120 detergent molecules bound to the membrane per molecule of lycopene inside. This binding ratio is likely overestimated owing to the fact that not all chromoplasts are filled with lycopene to the same extent and that there was likely binding of detergent to non-lycopene-containing membranes or membrane fragments in the preparation. Those caveats aside, the data are consistent with the hypothesis that the binding of detergent to the chromoplast membrane is what renders the chromoplasts more water-soluble. The chromoplasts are large; ~80% are retained on a 0.45 μm filter and appear to be of variable diameters up to as large as 6 μm. The lycopene molecules inside the chromoplast membrane form chiral centers that generate a distinctive cd spectrum. The fact that visible spectra of lycopene-containing chloroplasts in water suspensions and in SDS are very similar suggests that the organization of the lycopene molecules remains similar in the two systems. Solubility behavior of watermelon chromoplasts in SDS is more consistent with an interaction of the detergent with the chromoplast membrane. As is seen in other systems (*28*), the detergent−membrane interaction involves the interaction of SDS monomers binding to the chromoplast membrane rather than the involvement of detergent micelles. The interaction of SDS with the membrane amphiphiles would allow the integrity of the chiral aggregate of the lycopene inside the membrane to be maintained.

The physical nature of this interaction between watermelon chromoplasts and SDS is quite different from that observed by Takagi et al. (*29*−*31*) between free lutein and SDS. The comprehensive work of Takagi and co-workers describes a complex between lutein and SDS that exists at low SDS concentrations and/or at high ionic strengths. The size of the complex becomes larger with a decrease of SDS concentration and with an increase in salt concentration. The cd spectrum of the lutein changes with added amounts of SDS. A card-pack structure of a chiral nature was proposed as a model for the lutein aggregate. As SDS bound to lutein, the model was predicted to undergo an inversion to a second card-pack form. Additional amounts of SDS to excess levels resulted in disruption of the inverted card-pack form to physically smaller aggregates. Similar behavior was observed in SDS for zeaxanthin, a structural isomer of lutein, and thus more polar than lycopene (*32*).

Obviously, our lycopene preparation does not behave in a manner similar to lutein and zeaxanthin. In the case of the latter two carotenoids, SDS interacts directly with the carotenoid whereas, in this present study, the detergent interacts with the membrane that surrounds the lycopene assembly. Unlike lutein, the structural integrity of the large, chiral aggregate of lycopene inside the membrane is maintained at all concentrations of SDS. On the other hand, the bathochromic spectral shift of lycopene inside the chromoplast when compared to that of its monomeric state in hexane suggests an intermediate-coupling exciton energy transfer (*33*). The bathochromic spectral shift of the aggregates of lycopene inside the watermelon chromoplast is consistent with a head-to-tail configuration of the monomers in the aggregate to form a so-called *J*-aggregate (*34*, *35*). Depending upon the production process, *J*-aggregates have been observed for both β-carotene (*34*) and lycopene (*7*) when each was microencapsulated into a carotenoid nanoparticle. This type of behavior is consistent with the structural model proposed by Wang et al. (*36*) for lycopene in water-miscible solvents whereby both *H*-type and *J*-type aggregation of the molecules exist in the supramolecular structure. Therefore, subtle changes in the state of aggregation can cause either hypsochromic or bathochromic shifts in the spectrum of the complex as its aggregation state changes.

By nature of its two hydrophilic ends, lutein might be expected to disperse more readily into higher concentrations of SDS to form a mixed micelle with the detergent much in the same manner as cholesterol forms mixed micelles with phospholipids and detergents (*37*). Conversely, lycopene and β-carotene have no hydrophilic constituents, and the interaction between the carotenoid and the detergent might not be expected to be able to create a soluble assembly of detergent molecules about the carotenoid chiral aggregate. Additionally, we see no evidence of interaction between carotenoid and SDS micelles. Such behavior would explain the insolubility of lycopene in

SDS as evidenced by the behavior of commercial lycopene treated with SDS or lycopene from over-ripe watermelon where the chromoplast membranes are postulated to be partially or totally broken down leaving only the lycopene chiral aggregate to interact with the detergent.

The fact that the complex between SDS and lycopene-containing chromoplasts obeys Beer's law suggests that this system potentially can be employed for extracting and spectrally quantifying these carotenoids in selected systems. Such an application depends on (i) the capacity of the extracting medium to completely extract the compound of interest out of its natural matrix, (ii) the ability to differentiate the absorbance of the compound of interest from other potentially interfering absorbances, and (iii) the system obeying Beer's law. These requirements can likely be met by the SDS system in only limited cases. For example, aqueous SDS was unable to extract lycopene from processed tomato products and is therefore of no use for this food system. Tissues that contain numbers of carotenoids or other compounds whose absorption spectra overlap with that of the compound to be quantified will also not be amenable to this system. Despite these limitations, preliminary results with a number of varieties of watermelon and cantaloupe suggest that the method is applicable for lycopene quantification in watermelon tissues and β-carotene quantification in cantaloupe tissues.

ABBREVIATIONS USED

SDS, sodium dodecyl sulfate; HPLC, high-performance liquid chromatography; CMC, critical micelle concentration; CD, circular dichroism;

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