Photoisomerization of β-Carotene by Photosensitization with Chlorophyll Derivatives as Sensitizers†

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The ability of chlorophyll compounds, naturally present in extracts of green vegetables, to sensitize the photoisomerization of *all-trans*-β-carotene was investigated. Eight individual chlorophyll derivatives were evaluated for their ability to induce the photoisomerization of *all-trans*-β-carotene. All of the derivatives were found to act as sensitizers in the photoisomerization of *all-trans*-β-carotene. Additionally, *all-trans*-β-carotene added to vegetable extracts was photoisomerized. The final isomer distribution was similar to the equilibrium mixtures found using individual sensitizers. Isomerization reactions induced by chlorophyll may explain the presence of high percentages of cis isomers measured in photosynthetic tissues. Given the susceptibility of carotenoids to isomerize in the presence of chlorophyll derivatives, extra measures should be taken to avoid any exposure to light when handling extracts of plant tissue containing small quantities of chlorophyll. The results of these findings question whether the cis isomers in foods and biological tissues are naturally present or formed artifactually.

Keywords: *Isomerization; β-carotene cis isomers; chlorophyll derivatives; photosensitizers*

INTRODUCTION

Feeding studies in rats suggest that the two prominent cis isomers of β-carotene (13-*cis*-β-carotene and 9-*cis*-β-carotene) have lower provitamin A activities than *all-trans*-β-carotene (Sweeney and Marsh, 1973). Cis isomers of β-carotene have been detected in a variety of fruit and vegetables (Panalaks and Murray, 1970; Sweeney and Marsh, 1971; Chandler and Schwartz, 1987; Quackenbush, 1987). Table 1 lists β-carotene isomer distributions reported for several fruits and vegetables. Using open column chromatography, Sweeney and Marsh (1971) reported that green vegetables have a greater amount of 9-*cis*-β-carotene than 13-*cis*-β-carotene and that for other fruits and vegetables the reverse is usually seen. As shown in Table 1, this trend of greater amounts of 9-*cis*-β-carotene than other cis isomers in green vegetables was also reported when HPLC analyses were performed (Chandler and Schwartz, 1987).

Jensen et al. (1982) reported that chlorophyll *a* sensitized the photoisomerization of *all-trans*-β-carotene. Two times more 9-*cis*-β-carotene than 13-*cis*-β-carotene was formed, and small amounts of 15-*cis*-β-carotene were observed. The predominant cis isomer found in chlorophyll-containing vegetables is the same cis isomer that is formed to the largest extent in the chlorophyll-sensitized reaction. Although the photoisomerization reactions were performed in solution, questions remain regarding the natural presence or artifactual formation of cis isomers in chlorophyll-containing plant tissue extracts.

Ashikawa et al. (1986) investigated photoisomerization in plant tissue and thylakoid membranes. When the membranes were exposed to light, there was a reversible decrease in the relative amounts of 15-*cis*-β-carotene and 13-*cis*-β-carotene. The 9-cis isomer remained unchanged. Both red light and white light produced the same results, indicating chlorophyll sensitization may have led to these changes in isomer forms. On the other hand, these results suggest that the photoisomerization of *all-trans*-β-carotene into 9-*cis*-β-carotene does not occur in the tissues. Thus, the detection of this isomer may be an artifact of photoisomerization occurring during the extraction process.

Table 1. β-Carotene Isomer Distribution from Extracts of Fresh Vegetables[a]

vegetable	percentages of cis/trans isomers		
	13-cis	all-trans	9-cis
carrot	0.0	100.0	0.0
sweet potato	0.0	100.0	0.0
squash	15.3	75.0	9.7
spinach	8.8	80.4	10.8
cucumber	10.5	74.9	14.5

[a] Chandler and Schwartz (1987).

While there is usually 3 times more chlorophyll *a* than chlorophyll *b*, the latter is also present in plant tissues (Lichtenthaler, 1987). In addition to chlorophyll *b*, derivatives of chlorophyll may be present in processed foods, including pheophytin *a* and *b*, pyropheophytin *a* and *b*, and zinc pheophytin *a* and *b* (Schwartz and Lorenzo, 1990). Various porphyrin derivatives were employed to photoisomerize retinal, and differences in isomer distributions were found for sensitizers below the triplet energy state of retinal (Jensen et al., 1989). However, the ability of native chlorophyll derivatives to act as sensitizers for the photoisomerization of β-carotene has not been explored.

The purpose of this investigation was to determine if various chlorophyll compounds in extracts of green vegetables can photoisomerize β-carotene. Eight chlorophyll derivatives were evaluated for their ability to induce photoisomerization of *all-trans*-β-carotene and influence isomerization of *all-trans*-β-carotene in vegetable extracts.

MATERIALS AND METHODS

Materials. β-Carotene (type I) was purchased from Sigma Chemical Co. (St. Louis, MO). Fresh spinach, fresh kale, and

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canned spinach were purchased from local sources. Vegetable extracts were filtered through a nylon 66 0.45 μm pore (3 mm diameter) filter (Fisher Scientific Co., Pittsburgh, PA). All samples and solutions were stored at −18 °C.

All solvents, hydrochloric acid, and anhydrous Na_2SO_4 were ACS certified grade (Fisher Scientific). Solvents used in mobile phases for liquid chromatography were filtered through a 1.0 μm pore (47 mm diameter) PTFE filter (Fisher Scientific). Crystalline zinc chloride was from Sigma Chemical, and ACS certified grade $Ca(OH)_2$ was purchased from Aldrich Chemical Co. (Milwaukee, WI).

Fluorescent lights for illuminating samples were either General Electric (Cleveland, OH) cool white no. F15T8-CW or Philips Lighting Co. (Somerset, NJ) gold F40GO lamps. A light meter (840006) from Sperscientific Ltd. (Tempe, AZ) was used to measure light intensity.

Chromatographic Apparatus. The chromatographic apparatus includes a Waters Model 501 pump and a U6K injector (Water Associates, Milford, MA). A Waters 990 photodiode array detector equipped with NEC Powermate SX/20 computer and chromatography software was used to obtain and store absorption spectra and chromatograms. Chromatograms were monitored at 410 nm for the analysis of β-carotene separations. The wavelength 658 nm was used to monitor the separation of all chlorophyll derivatives except for the zinc pheophytins, where 643 nm was used to enhance detection sensitivity.

Separation of *cis/trans*-β-Carotene Isomers. For the analysis of β-carotene isomer distributions, a $Ca(OH)_2$-packed column, as described by Tsukida et al. (1982), with a mobile phase of hexanes:acetone (99.2:0.8 v/v) at 0.7 mL/min was used. Quantification was performed following previously reported procedures (O'Neil et al., 1991).

all-trans-β-Carotene was purified using a preparative $Ca(OH)_2$-packed column (500 × 9.4 mm) with a mobile phase of hexanes:acetone (99.2:0.8 v/v) at 2.2 mL/min. Following collection, the mobile phase was evaporated under N_2, and *all-trans*-β-carotene was redissolved in hexanes. The concentration was determined using a reported absorptivity constant of $E^{1\%}_{cm} = 2592$ in hexane at 453 nm (Isler et al., 1956).

Isolation of Chlorophyll Derivatives. The extraction of chlorophyll compounds from tissues was performed with acetone using the method of Canjura and Schwartz (1991). Chlorophyll *a* and *b* were obtained from fresh spinach extracts. Pheophytin *a* and *b* were obtained by dilute acid treatment of extracts (Vernon, 1960). Pyropheophytin *a* and *b* were extracted from canned spinach (Schwartz et al., 1981). Zinc pheophytin *a* and *b* were formed by complexing pheophytin *a* and *b* with zinc (Jones et al., 1968).

The mixtures of *a* and *b* derivatives were transferred to hexanes and purified using HPLC. Most of the chlorophyll derivatives were separated using a Zorbax silica column (4.6 × 250 mm) (MacMod Analytical, Chadds Ford, PA) with a mobile phase of 2-propanol:hexanes (1.7:98.3 v/v) at 1.5 mL/min (Canjura and Schwartz, 1991). Better separation of pyropheophytins was achieved with a reverse phase Zorbax ODS column (4.6 × 250 mm) and a mobile phase of ethyl acetate:methanol:water (50:37.5:12.5 v/v/v) at 1.5 mL/min (Schwartz and von Elbe, 1983).

Following the collection of individual derivatives from HPLC separations, solvents were evaporated under N_2 and the derivatives were redissolved in 2-propanol:hexanes (0.25:99.75 v/v). Concentrations were determined using reported specific absorption coefficients (Lichtenthaler, 1987; Jones et al., 1968). Coefficients for pheophytins were used for the determination of pyropheophytin *a* and *b* concentrations (Schwartz and von Elbe, 1983).

Conditions for Photoisomerization Experiments. The photoisomerization experiments were performed by exposing solutions to two fluorescent white lamps. The solutions and lamps were in an enclosed box to eliminate other sources of light. The light intensity at the surface of sample test tubes was 3000 lx. To control the temperature and minimize thermal isomerization, the box was in a cold room at 12 ± 1 °C. Solutions were kept under a N_2 atmosphere.

To simulate extraction conditions more accurately, a set of experiments with spinach extracts were also performed under normal extraction conditions. Gold lamps were used because carotenoids are often handled under these lighting conditions. These lamps filter out wavelengths where most carotenoids absorb visible light. The light intensity at the surface of sample test tubes was 56.0 lx. These experiments were carried out at room temperature (24 ± 2 °C). Experiments were performed as two replicates each with duplicate HPLC analyses. Control samples wrapped in foil were also prepared and analyzed.

Investigation of Chlorophyll *a* as Photosensitizer. Equilibrium isomer distributions with and without sensitizer were determined. *all-trans*-β-Carotene (2×10^{-4} M) was exposed to white light, and the extent of isomerization was measured at 2, 24, and 48 h. Following 48 h of exposure, chlorophyll *a* (0.5×10^{-5} M) was added, the samples were reexposed to light, and the extent of isomerization was measured at 2, 24, and 48 h.

The effect of sensitizer concentration on the extent of isomerization was determined by combining *all-trans*-β-carotene (1×10^{-4} M) with chlorophyll *a* at different concentrations [(0.02×10^{-5})–(20×10^{-5}) M]. These samples were exposed to white light for 2 h, and the extent of isomerization was measured as percent 9-*cis*-β-carotene in the final mixture.

Evaluation of Chlorophyll Derivatives as Photosensitizers. The following chlorophyll derivatives were evaluated as photosensitizers: chlorophyll *a*, chlorophyll *b*, pheophytin *a*, pheophytin *b*, pyropheophytin *a*, pyropheophytin *b*, zinc pheophytin *a*, and zinc pheophytin *b*. The isolated chlorophyll derivatives (2×10^{-5} M) and purified *all-trans*-β-carotene (1×10^{-4} M) were combined and exposed to white light for 2 h. The isomer distribution following exposure was measured. Additional controls without chlorophyll derivatives were also prepared and analyzed.

Isomerization of *all-trans*-β-Carotene in Vegetable Extracts. Fresh spinach and kale were extracted under gold lighting. For extraction, tissues (75 g) were pureed with H_2O (100 mL). Fifteen grams of puree was mixed with 30 mL of methanol for 2 min and filtered through Whatman no. 1 and no. 42 filter paper. The filter cake was suspended with 25 mL of acetone:hexanes (1:1 v/v), filtered, and reextracted (2×) with acetone:hexanes (1:1 v/v). Water (25 mL) was added, the epilayer removed, and the aqueous layer extracted twice with 30 mL of ethyl ether. The ether extracts were combined with the epilayer, washed three times with 25 mL of H_2O, dried over anhydrous sodium sulfate, and filtered through Whatman no. 1 filter paper. The extract was evaporated and redissolved in 50 mL of hexanes.

all-trans-β-Carotene (2.5×10^{-5} M) was added to spinach or kale extracts (containing 1×10^{-5} M *all-trans*-β-carotene and 2×10^{-4} M chlorophylls). Samples were exposed to white light for 2 h, and HPLC analyses to determine isomer changes were performed. Additionally, spinach extracts with added *all-trans*-β-carotene were exposed to gold light for 2 and 4 h. Gold lighting was used to simulate extraction conditions.

Saponification during Extraction of Pigments. Extraction of fresh spinach with KOH present to degrade chlorophylls was performed. In the dark, whole spinach leaves (10 g) were pureed with 30 mL of saturated methanolic KOH and held for 7.5 min and hexanes (20 mL) were added to extract carotenes. From the epilayer, approximately 10 mL was removed and resaponified for 2 h by adding 10 mL of ethyl ether and 25 mL of methanolic KOH. The hexanes layer was washed three times with H_2O (15 mL) and dried over Na_2SO_4. Absorption spectra of the extracts were obtained, using a Shimadzu recording spectrophotometer UV-240 (Shimadzu Corp., Kyoto, Japan), to ensure the removal of chlorophylls. Isomer distributions of extracts were measured by HPLC.

Statistical Methods. Experiments were performed as two replicates each with duplicate HPLC analyses. An analysis of variance (ANOVA) was used to evaluate differences (SAS, 1982).

RESULTS AND DISCUSSION

Investigation of Chlorophyll *a* as Photosensitizer. Figure 1 illustrates typical chromatograms show-

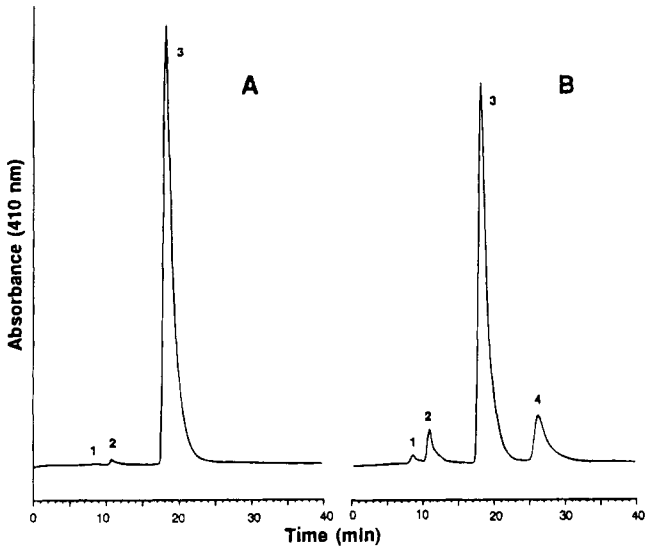

Figure 1. Chromatographic separation of β-carotene cis/trans isomers prior to (A) and following (B) the sensitized photoisomerization of *all-trans*-β-carotene. Peak identification: 1 = 15-*cis*-β-carotene, 2 = 13-*cis*-β-carotene, 3 = *all-trans*-β-carotene, and 4 = 9-*cis*-β-carotene.

Table 2. Changes in β-Carotene Isomer Distribution over Time

		percentage of cis/trans β-carotene isomers		
sample	time	13-cis	all-trans	9-cis
without chlorophyll *a*[a]	0	3.0	95	1.5
	2 h	3.1	96	1.5
	1 day	7.2	88	4.9
	2 days	6.5	88	5.1
with chlorophyll *a*[b]	0	6.5	88	5.1
	2 h	5.4	81	14
	1 day	4.9	83	12
	2 days	5.2	81	14

[a] Changes in isomer distribution from exposure to light without chlorophyll *a* present. [b] Changes in isomer distribution after chlorophyll *a* was added. Time zero was β-carotene solution previously equilibrated to lighted environment.

ing the separation of β-carotene isomers prior to and following chlorophyll-sensitized photoisomerization of *all-trans*-β-carotene. The chromatograms illustrate that photoisomerization of β-carotene occurred in the presence of sensitizer and that the cis isomers detected in photoisomerized samples included 9-*cis*-, 13-*cis*-, and 15-*cis*-β-carotene. The predominant cis isomer formed under these conditions was 9-*cis*-β-carotene. The starting material for the photoisomerization experiments was greater than 99% *all-trans*-β-carotene.

Table 2 lists the isomer distribution changes over time, when *all-trans*-β-carotene was exposed to light with and without chlorophyll *a* as sensitizer. After 2 h of exposure to light with no sensitizer present, there was not a significant change in isomer distribution. After 24 h of exposure to white light, there was a small increase in the overall percentage of isomers formed, but with further exposure to light there was not an observable change.

After the addition of chlorophyll *a*, photoisomerization occurred within a few hours and an equilibrium mixture was reached. Additional exposure to light (over 2 days) caused no significant change in isomer distribution. Thus, photoisomerization initiated by the sensitizer reaches an equilibrium within hours, while the isomerization that occurs with exposure to light alone required a longer period of time. Furthermore, the isomerization

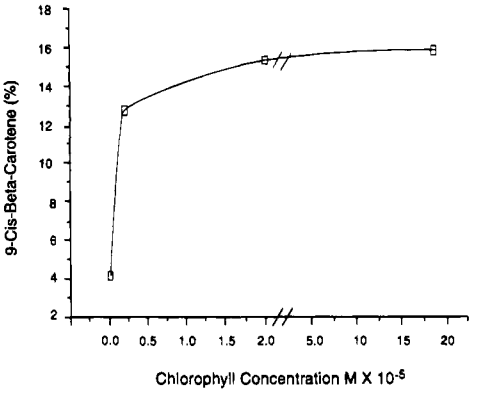

Figure 2. Effect of chlorophyll *a* concentration on the extent of photoisomerization of *all-trans*-β-carotene into 9-*cis*-β-carotene.

that occurs with sensitizer present results in a significantly greater quantity of cis isomers. The isomer distributions also differed in that while light without sensitizer produced relatively similar amounts of 13-*cis*-β-carotene and 9-*cis*-β-carotene, however, the isomerization with sensitizer led to the formation of predominately higher concentrations of 9-*cis*-β-carotene.

Effect of Chlorophyll *a* Concentration. Figure 2 shows the effect of chlorophyll *a* concentration on the extent of photoisomerization. Since 9-*cis*-β-carotene was the predominant cis isomer detected from chlorophyll *a*-sensitized photoisomerization, the extent of isomerization was measured as the percentage of 9-*cis*-β-carotene in the final mixture of isomers. A limiting concentration of chlorophyll *a* was found necessary to initiate isomerization. Because the mechanism of sensitized photoisomerization is likely via direct energy transfer, molecular interaction of excited sensitizer with β-carotene must occur. Therefore, the reaction is diffusion limited. If the lifetime of triplet state chlorophyll is less than the time required to interact with a β-carotene molecule, then the probability of energy transfer and subsequent β-carotene photoisomerization is minimal.

Because increasing the concentration of chlorophyll *a* beyond 2.0×10^{-5} M does not significantly increase the extent of isomerization, this concentration was selected to be used for the evaluation of all other chlorophyll derivatives. It should be noted that chlorophyll concentrations in extracts of photosynthetic tissues would be much higher than that required to initiate photoisomerization. On the other hand, extracts of yellow/orange vegetables may contain contaminating amounts of chlorophylls from root tip surface chlorophyll or small amounts of leafy material, which may or may not reach the requisite concentration to induce photoisomerization if exposed to light.

Evaluation of Chlorophyll Derivatives as Photosensitizers. Table 3 compares the ability of various chlorophyll derivatives to photoisomerize *all-trans*-β-carotene. The isomer distribution following exposure of *all-trans*-β-carotene to light in the presence of chlorophyll *a* compares well with the results of Jensen et al. (1982). All of the derivatives tested were able to act as sensitizers and the 9-cis isomer is the predominate cis isomer produced.

There were no significant differences in isomeric composition between the derivatives, with the exception of chlorophyll *a*, having slightly less 9-*cis*-β-carotene, and pheophytin *b*, having a higher percentage of 9-*cis*-β-carotene. The chlorophyll *a* samples had more *all-*

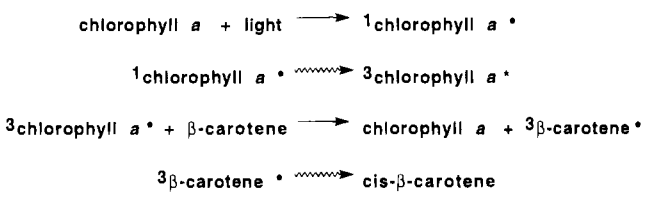

Figure 3. Proposed mechanism for the photoisomerization of β-carotene.

Table 3. Photoisomerization of *all-trans*-β-Carotene (ATBC) with Different Chlorophyll Derivatives as Sensitizers[a]

	percentages of cis/trans isomers			
	15-cis	13-cis	all-trans	9-cis
initial ATBC	0.01 ± 0.01	0.9 ± 0.2	99.1 ± 0.2	0.00 ± 0.01
sensitizer				
chlorophyll *a*	0.88 ± 0.08	5.2 ± 0.2	81 ± 0.9	13 ± 1
chlorophyll *b*	0.95 ± 0.06	5.7 ± 0.1	78 ± 0.3	15 ± 0.5
pheophytin *a*	0.80 ± 0.06	5.3 ± 0.4	79 ± 1	15 ± 0.6
pheophytin *b*	0.87 ± 0.03	5.9 ± 0.04	77 ± 0.2	16 ± 0.2
pyropheophytin *a*	0.93 ± 0.10	5.3 ± 0.5	80 ± 1	14 ± 1
pyropheophytin *b*	0.98 ± 0.04	5.9 ± 0.3	79 ± 0.4	14 ± 0.3
Zn-pheophytin *a*	1.01 ± 0.02	5.7 ± 0.2	78 ± 0.5	15 ± 0.3
Zn-pheophytin *b*	1.03 ± 0.02	6.0 ± 0.2	78 ± 0.7	15 ± 0.5
controls				
no light	0.7 ± 0.3	3.2 ± 1.5	95 ± 3	1.2 ± 0.9
no sensitizer	1.5 ± 0.8	8.4 ± 4.5	88 ± 7	2.3 ± 1.5

[a] Average ± standard deviations.

trans-β-carotene than other photoisomerized samples as a result of less 9-*cis*-β-carotene being formed. While these differences in carotene content were statistically significant ($p < 0.05$), the overall change is minimal. Among all the chlorophyll derivatives tested for their ability to photoisomerize β-carotene, the relative percentages of isomers formed are similar.

Comparing these isomer distributions to controls that were not exposed to light, significant differences in the percentages of *all-trans*-β-carotene and 9-*cis*-β-carotene were observed. These results confirm that isomerization occurred through a light-initiated mechanism. The increases in the 15-cis and 13-cis isomers with sensitized photoisomerization were not as large as for the 9-cis isomer. For several of the samples, there were no significant differences between the photoisomerized and control samples in the amounts of 15-*cis*- and 13-*cis*-β-carotene.

Control samples exposed to light without sensitizers were also prepared. These samples varied slightly in isomer distribution from experiment to experiment (possibly due to room temperature fluctuations); however, the predominant cis isomer detected was 13-*cis*-β-carotene, and only small quantities of 9-*cis*-β-carotene were observed. Thus, light alone did not photoisomerize *all-trans*-β-carotene into 9-*cis*-β-carotene to the extent observed when chlorophyll compounds are present. These findings support the mechanism of isomerization proposed by Jensen et al. (1982). The isomerization is thought to occur following the quenching of excited state triplet by β-carotene. Figure 3 shows the steps involved in the mechanism.

Except for samples containing zinc pheophytins, exposure to light for 2 h did not lead to losses in total β-carotene. Differences in the concentration of total β-carotene between the exposed samples and the samples not exposed to light were minimal. The losses in samples containing zinc pheophytin were not large (average <10%) and likely due to contaminating metal ions acting as a catalyst for oxidation of carotene.

Isomerization of *all-trans*-β-Carotene in Vegetable Extracts. Table 4 lists the percentages of cis/trans isomers found in the extracts of fresh spinach and kale. The extracts were found to have more 9-*cis*-β-carotene than other predominant cis isomers. These included 13-*cis*-β-carotene and 15-*cis*-β-carotene. These findings are in agreement with previous reports (Sweeney and Marsh, 1971; Quackenbush, 1987; Chandler and Schwartz, 1987). A peak following the 13-*cis*-β-carotene peak was observed in the kale extracts and tentatively identified as di-cis isomers of β-carotene. This identification was based on absorption spectra and relative retention time in comparison to previous separations (O'Neil et al., 1991; Koyama et al., 1988).

Table 4. Effect of Light on the Isomerization of *all-trans*-β-Carotene (ATBC) Added to Vegetable Extracts[a]

vegetable/ conditions	sample	percentages of cis/trans isomers				
		15-cis	13-cis	di-cis	ATBC	9-cis
spinach/white light, 2 h	initial extract	1.5	6.8		81	11
	extract plus ATBC exposed to light	3.0[b]	6.4		77	13
	unexposed control	2.8[b]	5.0		85	6.9
kale/white light, 2 h	initial extract	0.18	3.7	4.1	79	13
	extract plus ATBC exposed to light	2.9	4.0	4.0[b]	75	14
	unexposed control	3.3	5.0	3.6[b]	80	8.2
spinach/gold light, 2 h	extract plus ATBC exposed to light	3.0[b]	6.4		81	9.3
	unexposed control	3.0[b]	5.1		86	5.5
spinach/gold light, 4 h	extract plus ATBC exposed to light	3.2	6.7		78	12
	unexposed control	2.9	4.8		87	5.6

[a] Added β-carotene isomer distribution: 15-cis = 3.5, 13-cis = 3.0, all-trans = 92, and 9-cis = 1.5. [b] No significant difference between exposed and unexposed samples.

The results of adding *all-trans*-β-carotene to vegetable extracts and exposing these samples to light are also shown in Table 4. Evaluation of isomer distributions demonstrated that isomerization of *all-trans*-β-carotene to cis isomers occurred with light exposure. Adding *all-trans*-β-carotene decreased the percentage of cis isomers in the samples, but with exposure to light, a new equilibrium mixture with higher percentages of cis isomers was achieved. The percentage of the β-carotene in the 9-cis configuration was similar to that found with samples subjected to sensitized photoisomerization. Differences between the light-exposed solutions and the unexposed solutions were observed for *all-trans*-β-carotene and 9-*cis*-β-carotene, confirming that significant changes occurred with light exposure. Thus, the predominant isomer formed during photoisomerization in the vegetable extracts was the 9-cis isomer.

Gold lighting is used by this laboratory and by others to filter out visible light absorbed by carotenoids. Eliminating the absorption aids in limiting photodegradation. However, the gold filter does not completely block the wavelengths where chlorophyll compounds absorb. Therefore, gold lighting was also investigated as a potential initiator of sensitized photoisomerization. The results of these experiments are also shown in Table 4. After 2 h of exposure, changes in the isomer distribution were found, but the isomerization was not as great as was observed with exposure to white light. Within 4 h, there were no differences between the isomer distribution in samples exposed to gold light and the photoisomerized samples from white light exposure. Thus, gold lighting does provide some protection against the sensitized photoisomerization. However, this lighting will not prevent such reactions, only slow the rate of formation. Most likely the slower reaction rate is caused by a decrease in light intensity when gold lights are the energy source.

Saponification during Extraction of Pigments. The fact that *all-trans*-β-carotene was photoisomerized under extraction conditions prompted the question as to whether the isomers detected in extracts of photoisomerized tissues were actually within the native tissues or formed during extraction. In order to decrease the opportunity for photoisomerization, fresh spinach was extracted with methanolic KOH in the dark. The base reacts with the chlorophylls to produce polar products, thereby degrading the photosensitizer. Slightly lower percentages of 9-*cis*-β-carotene were detected with this procedure (8.6%) compared to the percentages with sensitized extracts (11%). However, this method of extraction did not eliminate the detection of cis isomers. Thus, these findings suggest that cis isomers, particularly the relatively higher percentage of the 9-cis form, may be naturally present in the photosynthetic tissues.

Ashikawa et al. (1986), in determining light-induced changes in β-carotene isomers present in thylakoid membranes, presented evidence to support the belief that cis isomers are naturally present in photosynthetic tissue. These authors noted that extracts of different sources [photosystem I (PSI), photosystem II (PSII), thylakoid membranes, and intact cells] differed in isomer distribution. Additional evidence included the fact that the calculated isomer distribution for thylakoid membranes, based on the analysis of PSI and PSII separately, was similar to the results actually measured.

Thus, on the basis of the fact that cis isomers were found even with the elimination of chlorophylls in the early stages of extraction and the evidence presented by Ashikawa et al. (1986), appreciable levels of cis isomers may actually be present in photosynthetic tissues. Futhermore, since the results of this study demonstrate that chlorophyll-sensitized photoisomerization can occur rapidly in vegetable extracts, extreme care to eliminate light should still be taken to ensure that the extraction process does not alter the endogenous isomer composition.

CONCLUSIONS

A variety of chlorophyll derivatives (chlorophyll *a* and *b*, pheophytin *a* and *b*, and zinc pheophytin *a* and *b* complexes) were found to act as effective photosensitizers inducing the formation of β-carotene cis isomers. These photochemically induced isomerization reactions show predominantly the formation of the 9-cis isomer when chlorophyll photosensitizers are present. The mechanism is believed to act via an excited state triplet of chlorophyll and its derivatives. Thus, isomerization reactions photosensitized by chlorophyll may explain the presence of high percentages of cis isomers measured in photosynthetic tissues. Given the susceptibility of carotenoids to isomerize in the presence of chlorophyll derivatives, extra measures should be taken to avoid any exposure to light when handling extracts of photosynthetic plants. The results of these findings question whether the cis isomers in foods and biological tissues are naturally present or formed artifactually.

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