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Collision Cross-Section Determination and Tandem Mass Spectrometric Analysis of Isomeric Carotenoids Using Electrospray Ion Mobility Time-of-Flight Mass Spectrometry

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Abstract

Carotenoids are natural pigments with provitamin A and antioxidant activities. Biosynthesized in plants as their all-trans isomers, carotenoids isomerize in solution and in humans to multiple cis isomers which can have different bioavailabilities and functions. Since separation and characterization of isomeric carotenoids using HPLC or LC-MS-MS is time consuming, the potential for ion mobility mass spectrometry (IM-MS) to resolve and characterize carotenoid isomers rapidly without chromatography was investigated using travelling-wave ion mobility spectrometry on a quadrupole time-of-flight mass spectrometer. The all-trans isomers of lycopene and β -carotene were separated by several milliseconds from the cis-isomers which were detected as partially overlapping peaks. The collision cross-section values of these carotenoid isomers were determined using IM-MS to be 180 Å² and 236 Å² for *cis*-lycopenes and all-*trans*-lycopene, and 181 Å² and 225 Å² for *cis*- β -carotene and all-*trans*- β -carotene, respectively. Collision-induced dissociation MS-MS of ion mobility-resolved isomers indicated that cis and all-trans carotenoid isomers can be distinguished by their fragmentation patterns. Previous MS-MS studies of *cis*- and all *trans*-carotenoids had suggested that they produced identical tandem mass spectra, but this appears to have been the result of isomerization during ionization. Introduction of specific cis or trans isomers by infusion or HPLC resulted in cis/trans isomerization in the ion source during electrospray, and the relative levels of cis carotenoids forming in the ion source compared to the all-trans isomers were temperature dependent.

Keywords

ion mobility; collision cross-section; mass spectrometry; electrospray; carotenoids; lycopene; β -carotene

Carotenoids are botanical pigments that have provitamin A and antioxidant activities^{1–3}. Among the most abundant dietary carotenoids, β -carotene can be converted to retinol (vitamin A) during intestinal absorption, and lycopene is under investigation as a prostate cancer prevention agent.^{1–3} Biosynthesized in plants as the all-trans isomers, dietary

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carotenoids can form multiple *cis* isomers when exposed to heat or light, and these isomers have been reported to have different bioavailabilities.^{4–6} The planar chemical structures of all-*trans*-lycopene, all-*trans*- β -carotene and two of their thermodynamically favored *cis* isomers are shown in Figure 1.

Since carotenoids are too labile to be analyzed using GC-MS,^{7–10} HPLC and LC-MS analyses using special C₃₀ stationary phases have become routine to separate the various *cis* and *trans* isomers.^{11–17} Effective at resolving geometric isomers of dietary carotenoids in biomedical matrices, carotenoid separations using C₃₀ columns can require up to 90 min per analysis.¹⁸ Since faster alternatives to chromatography would be most helpful for the analysis of carotenoid isomers in biomedical samples, the feasibility of using ion mobility mass spectrometry (IM-MS) for this purpose was investigated.

Ion mobility spectrometry is the gas-phase separation of ions in an electric field on the basis of size and shape. When coupled with mass spectrometry, a dimension of high speed separation can be added to mass spectrometric analysis.^{19–21} Due to thermal instability, carotenoids might isomerize during the ionization process. Therefore, the possibility of in-source *cis/trans* isomerization of carotenoids was also investigated during this study. Geometric isomers of natural products including *cis/trans* carotenoids have been reported to produce identical tandem mass spectra during collision-induced dissociation and product ion analysis.^{9, 22–25} With the gas phase separation of traveling wave ion mobility on a quadrupole time-of-flight mass spectrometer, we were able to revisit the tandem mass spectrometry of *cis/trans* carotenoids.

EXPERIMENTAL SECTION

Chemicals and Reagents

All-*trans* lycopene, all-*trans*- β -carotene and poly-DL-alanine were purchased from Sigma-Aldrich (St. Louis, MO). The isomeric purity of each carotenoid standard was verified using reversed-phase LC-MS-MS with a YMC (Wilmington, NC) C₃₀ carotenoid column (4.6×250 mm, 3 μ m) as reported previously.¹⁶ Working solutions of lycopene and β -carotene were prepared immediately before use from solid standards to minimize isomerization and degradation. Mixtures of *cis* and *trans* isomers of each carotenoid were prepared by dissolving the all-*trans* forms in methylene chloride in a sealed vial and allowing isomerization to occur during exposure to fluorescent lighting for 24 h. Methanol, acetonitrile, methyl-*tert*-butyl ether (MTBE), and formic acid (all HPLC grade) and methylene chloride (ACS reagent grade) were purchased from Thermo Fisher (Hanover Park, IL). Deionized water was prepared using a Millipore (Bedford, MA) Milli-Q system.

Effect of Electrospray on Isomerization

Positive ion electrospray ion mobility time-of-flight mass spectrometry was carried out using a Waters Synapt HDMS quadrupole time-of-flight hybrid mass spectrometer (Manchester, UK) equipped with traveling-wave ion mobility spectrometry (TWIMS). All-*trans* lycopene or all-*trans*- β -carotene (20 μ g/mL in acetonitrile/MTBE, 1:1, v/v) or mixtures of the respective *cis/trans* isomers were infused into the ion source at a flow rate of 10 μ L/min. Optimum positive-ion electrospray and ion mobility separation conditions included the following: capillary voltage, 4.0 kV; sampling cone voltage, 26.0 V; extraction cone voltage, 4.0 V; ion source temperature, 100 °C; cone gas flow rate, 20 L/h; desolvation gas flow rate, 500 L/h; trap collision energy, 6.0 V; transfer collision energy, 4.0 V; trap gas flow rate, 5.0 mL/min; ion mobility gas flow rate, 20.0 mL/min (~0.45 mBar); wave velocity, 300 m/s; and wave height, ramping from 5.0 V to 12.0 V. The effect of desolvation gas temperature was investigated from 50 to 300 °C with increments of 50 °C. The duty cycle of the time-of-flight mass analyzer was optimized for the analysis of carotenoids at *m/z* 536.4 during data

acquisition to maximize signal intensity throughout the range of desolvation gas temperatures. Data were acquired using MassLynx (V4.1, Waters) and processed using Driftscope (V2.1, Waters) software.

Effect of Collision-Induced Dissociation on Isomerization

All-*trans* lycopene or all-*trans* -carotene (20 µg/mL in acetonitrile/MTBE, 1:1, v/v) were infused into the electrospray ion source at 10 µL/min along with 100 µL/min methanol as a make-up solvent. The ionization parameters were identical to those described above except that a capillary voltage of 5 kV and a desolvation gas temperature of 500 °C were applied. The ion mobility conditions included a nitrogen flow of 23 mL/min at ~0.51 mBar, a wave velocity of 300 m/s and a ramping of wave height from 7.0 V to 12.0 V. Positive ion collision-induced dissociation (CID) product ion tandem mass spectra were recorded for molecular ions of the *cis*- and all-*trans*-carotenoids of *m/z* 536.4 exiting from the ion mobility cell. Argon was used as the collision gas at energies of 20, 25, 27.5, 30, 32.5, 35, 37.5, 40, 42.5, or 45 V.

LC-MS-IM-CID-TOF MS

A Waters (Milford, MA) 2695 HPLC was interfaced to the Synapt HDMS system, and chromatographic separation of all-*trans* lycopene or all-*trans* -carotene and their corresponding *cis*-isomers was carried out using a C₃₀ carotenoid column (2.1×250 mm, 3 µm) with a 45 min step gradient from methanol to MTBE at a flow rate of 0.30 mL/min as follows: 0 min, 65% methanol; 30 min, 40% methanol; 35.1 min, 65% methanol. A 20 µL aliquot of a mixture of lycopene and -carotene *cis/trans* isomers containing ~95 µg/mL lycopene and ~50 µg/mL -carotene in acetonitrile/MTBE (1:1, v/v) was injected onto the column. Using positive ion electrospray ionization, ions of *m/z* 536.4 were selected and separated by using TWIMS, and CID was carried out in the transfer collision cell at 35 V for lycopene and 40 V for -carotene. Product ion tandem mass spectra of the molecular ions of lycopene or -carotene were recorded as well as their HPLC retention times and ion mobility drift times.

Collision Cross-Section Measurements

The collision cross-sections of lycopene and -carotene were experimentally determined using the Synapt HDMS system based on the method of Williams *et al.*,²⁶ except that poly-DL-alanine was used for calibration instead of tryptic hemoglobin peptides. Poly-DL-alanine (1 mg/mL) was prepared in acetonitrile/0.1% aqueous formic acid (1:1, v/v) and infused into the ion source at 10 µL/min with 100 µL/min methanol as a make-up solvent. The electrospray ion source conditions were identical to those used for the CID measurements described above. Using the calibration solution, the ion mobility separation parameters were optimized at IMS gas flow rates of 20 mL/min or 23 mL/min. The optimized ion mobility separation parameters at an IMS gas flow rate of 20 mL/min included a wave velocity of 300 m/s and a ramping wave height from 5.5 V to 12.0 V. For the 23 mL/min IMS gas flow rate, optimized operating conditions included a ramping wave height from 7.0 V to 12.0 V and a wave velocity of 300 m/s. All-*trans* lycopene and all-*trans* -carotene (20 µg/mL in acetonitrile/MTBE, 1:1, v/v) were each infused and analyzed using the optimized conditions. A programmable dynamic range enhancement lens was used for the response adjustment of poly-DL-alanine when necessary. Based on the collision cross-section values of poly-DL-alanine molecules in the literature,²⁷ TWIMS calibration curves were plotted for each IMS gas pressure and wave height combination, and the collision cross-sections of lycopene and -carotene isomers were determined using Driftscope software.

The theoretical collision cross-section values of all *trans*-lycopene, 5-*cis*-lycopene, all *trans*-carotene, and 13-*cis*-carotene were determined using the Tripos (St. Louis, MO)

molecular modeling program Sybyl-X (ver. 1.1.1). The minimum and maximum diameters of each molecule were determined at different molecular orientations, and the mean of these values was determined to estimate each collision cross-section.

RESULTS AND DISCUSSION

The isomeric purities of the all-*trans*-lycopene and all-*trans*- β -carotene standards in freshly prepared solutions were 92% as indicated by HPLC with mass spectrometric detection of the molecular ions of m/z 536.4 which comprised the base peak of each mass spectrum. The light-isomerized carotenoid solutions were analyzed using LC-MS, and the *cis*/all-*trans* ratios were 62%/38% and 14%/86% for lycopene and β -carotene, respectively. The isomeric carotenoid mixtures were infused into the electrospray ion source to facilitate tuning of the ion mobility separation conditions. IMS flow rates ranging from 5–28 mL/min were investigated for analysis of lycopene and β -carotene, and the best results were obtained at 20 mL/min. The optimum wave velocity was 300 m/s, although variations in the wave velocity produced only small changes in carotenoid isomer separation during IM-MS. The height of the traveling wave had the most impact on the ion mobility separation of carotenoid isomers, and ramping the wave height from 5 V to 12 V produced the best ion mobility separations.

As shown in Figure 2A, two lycopene peaks were observed during IM-MS with drift times differing by 3.6 ms for the isomers of m/z 536.4. Presumably, the first peak consisted of a mixture of *cis* isomers, and the linear all-*trans* isomer was detected as the second peak. To confirm which peak corresponded to the all-*trans* isomer, a fresh solution of all-*trans* lycopene was infused into the electrospray ion source. Instead of one peak corresponding to the all-*trans* isomer, two peaks were observed in the same ratio and with the same drift times as those observed for the isomeric lycopene mixture (Figure 2B). Similar results were obtained for the *cis* and *trans* isomers of β -carotene. This suggested that in-source isomerization of carotenoids was occurring prior to ion mobility separation.

To make certain that isomerically pure *cis*-lycopenes and *cis*- β -carotenes were entering the ion source, C₃₀ chromatographic separation of the *cis*/*trans* isomeric mixtures was carried out on-line with IM-MS (LC-IM-MS), and the ion mobility drift time spectra were recorded for various *cis*-isomers as well as the all-*trans* isomers of lycopene and β -carotene. The molecular ions of m/z 536.4 for each chromatographically resolved carotenoid isomer were analyzed using LC-IM-MS, and two peaks were observed for each carotenoid (Figure 3 and Supplemental Figure 1). These peaks were in the same *cis*/*trans* ratios as had been observed during infusion of mixtures of *cis* and *trans* isomers of β -carotene (Figure 3) or lycopene (Supplemental Figure 1). Regardless of the *cis*/*trans* ratio of lycopene or β -carotene entering the electrospray ion source, a constant ratio of isomers was formed in the source and detected using IM-MS.

Cis/*trans* isomerization of carotenoids is known to occur easily upon exposure to heat or light,^{8–9,15,17} but the process of molecular ion formation during electrospray might also contribute to *cis*/*trans* isomerization, especially since abstraction of a β -electron is likely to occur. Thermal isomerization to an equilibrium of *cis*/*trans* carotenoid isomers in the electrospray source might explain, at least in part, the mixture of isomers of m/z 536.4 for lycopene or β -carotene detected using IM-MS and LC-IM-MS regardless of the isomeric purity of the compound entering the ion source. To test this thermal isomerization hypothesis, the temperature of the desolvation gas used during electrospray was varied from 50 °C to 300 °C during sample infusion (note that desolvation gas temperatures below 50 °C or above 300 °C did not produce stable electrospray), and the relative amounts of *cis* and all-*trans* isomers were determined at each temperature using IM-MS. As shown by the drift time distributions in Figure 4 and the graphs in Figure 5, the relative abundance of the first ion

mobility peak compared to the second peak increased as the temperature in the ion source was increased. This observation is consistent with the hypothesis that the first peak in each drift time spectrum (Figures 2, 3 and 4) corresponds to the higher energy *cis* isomers, and the second IM-MS peak is the thermodynamically more stable all-*trans* isomer.^{6,28}

The collision cross-section, which characterizes the shape and conformation of a molecule in the gas phase, is proportional to the mobility of an ion during IM-MS.^{20,29–30} TWIMS calibration curves were generated by using Driftscope software for each IMS gas pressure and wave height combination. The calibration curves were $y = 192.65x^{0.6796}$ ($r^2=1.000$) and $y = 189.33x^{0.6890}$ ($r^2=1.000$) at IMS gas flow rates of 20 and 23 mL/min, respectively. The collision cross-section values of the *cis* and all-*trans* isomers of lycopene and β -carotene were determined using the standard curves, and the results are summarized in Table 1. Theoretical collision cross-sections were calculated for the all-*trans* isomers and some of the most abundant *cis* isomers of lycopene and β -carotene, and these results are shown in Table 1.

As indicated in Table 1, the lycopene isomers showed larger collision cross-sections than did the corresponding β -carotene peaks. Since lycopene and β -carotene are structural isomers, this difference was probably due to the acyclic, elongated structure of lycopene compared to the cyclic, β -ionone rings of β -carotene. The more compact *cis* isomers of each carotenoid probably produced the first peak during IM-MS, and the all-*trans* isomer should have a longer drift time corresponding to the second peak. The measured collision cross-section of the *cis* isomers and the all-*trans* isomer of lycopene were 180 Å² and 236 Å², respectively. For β -carotene, the measured collision cross-sections of the *cis* and all-*trans* isomers were 181 Å² and 225 Å², respectively. The IMS gas flow rate of 23 mL/min produced slightly smaller collision cross-section values for both sets of isomers than did the 20 mL/min flow rate suggesting that carotenoids become more compact as the IMS gas flow rate and pressure increases.

The theoretical collision cross-section values of lycopene and β -carotene were similar in magnitude to the measured values (Table 1). As expected, the β -carotene values were smaller than the corresponding lycopene values due to the presence of β -ionone rings in β -carotene, and the *cis* isomers had smaller collision cross-section values than did the corresponding all-*trans* isomers. For example, the theoretical collision cross-section of all-*trans*- β -carotene was 239 Å² compared with the larger value of 268 Å² for the acyclic all-*trans*-lycopene. Although IM-MS measurements could be made only on mixtures of *cis* isomers, theoretical collision cross-section values could be calculated for specific *cis* isomers. As an example of a carotenoid with a *cis* double bond located near one terminus, the collision cross-section of 5-*cis*-lycopene (Figure 1) was determined to be 232 Å² which was slightly smaller than that of all-*trans*-lycopene (Table 1). In contrast, the collision cross-section of 13-*cis*- β -carotene was only 125 Å² compared with the all-*trans* isomer value of 239 Å² due to the central location of the *cis* double bond in this isomer (Figure 1).

Previously, we reported that the product ion tandem mass spectra of *cis* and all-*trans* isomers of a particular carotenoid were identical, either because CID cannot produce distinguishing fragmentation of *cis*/*trans* isomers or because isomerization occurred during ionization, CID or at both steps of the analysis.⁹ Since IMS can separate *cis* and *trans* carotenoid ions after the ionization process, the use of IM-MS-MS provided an opportunity to determine if carotenoid geometrical isomers can be distinguished based on their fragmentation patterns produced during CID. The positive ion electrospray IM-MS-MS spectra of the *cis* and all-*trans* isomers of lycopene are shown in Figure 6. Although both geometrical isomers formed product ions of identical masses, the relative abundances of these ions varied considerably. All-*trans*-lycopene (drift time 8.0 ms) fragmented much more extensively than did the *cis*-

lycopene isomers (drift time 5.5 ms). The base peak in the tandem mass spectrum of all-*trans*-lycopene corresponded to an ion of m/z 69 which was formed by the loss of a terminal isoprene group (measured m/z 69.0706, theoretical m/z 69.0704; C_5H_9 M 2.9 ppm), whereas the base peak of the *cis* isomers was the molecular ion of m/z 536 (measured m/z 536.4405, theoretical m/z 536.4382; $C_{40}H_{56}$ M 4.3 ppm). An abundant ion of m/z 69 was observed in the tandem mass spectrum of *cis*-lycopenes at a relative abundance of 76% (measured m/z 69.0706, theoretical m/z 69.0704; C_5H_9 M 2.9 ppm), and the molecular ion of all-*trans*-lycopene was observed at a relative abundance of 4% (measured m/z 536.4405, theoretical m/z 536.4382; $C_{40}H_{56}$ M 4.3 ppm). Another feature that distinguished the IM-MS-MS spectra of geometrical isomers of lycopene was the product ion of m/z 467 (measured m/z 467.3663, theoretical m/z 467.3678; $C_{35}H_{47}$ M -3.2 ppm), formed by the elimination of a terminal isoprene moiety,³¹ which was observed at a relative abundance of 91% in the tandem mass spectrum of the *cis* isomers but at only 17% abundance in the tandem mass spectrum of all-*trans*-lycopene (Figure 5). The ion of m/z 444 which corresponds to the elimination of toluene³¹ was observed in the tandem mass spectrum of all-*trans*-lycopene (measured m/z 444.3765, theoretical m/z 444.3756; $C_{33}H_{48}$ M 2.0 ppm) but not in the tandem mass spectrum of the *cis*-lycopenes. Ions of m/z 521 (measured m/z 521.4144, theoretical m/z 521.4147; $C_{39}H_{53}$ M -0.6 ppm) and m/z 493 (measured m/z 493.3883, theoretical m/z 493.3834; $C_{37}H_{48}$ M 9.9 ppm) which correspond to losses of methyl and propyl radicals, respectively, were observed only in the tandem mass spectrum of the *cis*-lycopene isomers.

The *cis* and all-*trans* isomers of lycopene may be distinguished during IM-MS-MS based on their different drift times (all-*trans*-lycopene showed the longest drift time) and by the relative abundances of fragment ions of m/z 467, m/z 521 and m/z 493 (*cis*-lycopenes) and m/z 444 (all-*trans*-lycopene). The M^+ ions of *cis*-lycopenes fragment during collision-induced dissociation to form product ions of m/z 521 and m/z 493 whereas all-*trans*-lycopene either does not form these ions or produces them at much lower relative abundances. Furthermore, *cis*-lycopenes fragment during MS-MS to form an abundant ion of m/z 467 (91%) whereas the ion of m/z 467 in the tandem mass spectrum of all-*trans*-lycopene is detected at more than 5-fold lower abundance (17%). Therefore, the detection of ions of m/z 521 and m/z 493 and an abundant ion of m/z 467 in the IM-MS-MS spectrum of lycopene is characteristic of *cis*-lycopenes. The absence of ions of m/z 521 and m/z 493 in the IM-MS-MS spectrum of lycopene along with the detection of an ion of m/z 444, which is absent from the product ion tandem mass spectrum of *cis*-lycopene, would be characteristic of all-*trans*-lycopene.

To determine if the *cis* and all-*trans* isomers of β -carotene could be distinguished using IM-MS-MS, the positive ion tandem mass spectra of β -carotene isomers were obtained and are shown in Figure 7. The base peak of the tandem mass spectrum of all-*trans*- β -carotene was observed at m/z 444 and corresponded to the loss of toluene (measured m/z 444.3756, theoretical m/z 444.3756; $C_{33}H_{48}$ M 0 ppm). Since the ion of m/z 444 was observed at a relative abundance of only 11% in the tandem mass spectrum of the β -carotene *cis* isomers, the relative abundance of this ion may be used to distinguish the *cis* and all-*trans* β -carotene isomers. The base peak of the tandem mass spectrum of the *cis*- β -carotenes was the molecular ion of m/z 536 (measured m/z 536.4378, theoretical m/z 536.4382; C_5H_9 M -0.7 ppm). Although the *cis* isomers of β -carotene did not fragment as extensively as did all-*trans*- β -carotene, the ion of m/z 521 formed by the loss of a methyl radical (measured m/z 521.4119, theoretical m/z 521.4147; $C_{39}H_{53}$ M -5.4 ppm) was more abundant in the tandem mass spectrum of the *cis*- β -carotenes. In the IM-MS-MS analyses of the lycopene and β -carotene isomers, the ion of m/z 444 was more abundant in the tandem mass spectra of the all-*trans* isomers, and the ion of m/z 521 was more abundant in the tandem mass spectra of the *cis* isomers.

Although carotenoids may be distinguished based on their tandem mass spectra,³¹ both cis and trans carotenoids produce identical tandem mass spectra due to isomerization that occurs during the ionization process. Even though electrospray is one of the softest ionization techniques, we have shown that cis/trans isomerization of carotenoids still occurs during this ionization process. This in-source isomerization phenomenon probably explains why MS and MS/MS produces identical responses for all-trans and various cis isomers of retinoic acid³² or a particular carotenoid.¹⁶ When carrying out quantitative analysis, HPLC with absorbance detection responds differently to cis and trans isomers of carotenoids and retinoic acid, and therefore, separate standard curves are required for each geometric isomer. However, isomerization in the ion source appears to simplify quantitative analysis of these compounds when using LC-MS or LC-MS-MS, since one standard curve would be suitable for all geometric isomers of a particular compound.

When carotenoids are analyzed using IM-MS, our data indicate that the cis and trans isomers may be resolved and that they produce unique tandem mass spectra that may be used to distinguish the all-trans isomer from the cis isomers. Although HPLC may be used to separate geometric isomers of carotenoids, ionization techniques such as FAB³¹ and electrospray isomerize these compounds. Since the time course for ionization is probably ~0.1 s (assuming ~10 cc/s nebulizing gas and solvent vapor in a 1 cm² spray, the flow rate would be ~10 cm/s; and for a 1 cm path length, ~0.1 s would be required for sample to travel from the electrospray emitter to the sampling cone of the mass spectrometer), carotenoid isomerization might be complete by the time ions were extracted from the ion source and tandem mass spectra were obtained. However, IM-MS-MS requires only milliseconds for separation of isomers, fragmentation during CID and analysis, which is sufficient to preserve cis/trans isomeric information. Until carotenoid ionization can be achieved without cis/trans isomerization, the slow process of HPLC separation will still be necessary as part of the analysis of carotenoid cis/trans isomers in biomedical and biological samples.

CONCLUSIONS

The separation of cis and all-trans isomers of carotenoids was demonstrated using TWIMS with drift time separations of 1–4 ms. Because the cis isomers had collision cross-sections that were smaller than those of the corresponding all-trans isomers, they were detected first followed at a later drift time by the all-trans isomer. However, the various cis isomers could not be resolved using this particular instrument.

The use of LC-IM-MS-MS helped confirm that in-source isomerization of lycopene and β -carotene occurred during positive ion electrospray. This in-source isomerization was found to be temperature dependent. Although cis/trans isomerization could not be eliminated during electrospray, this temperature dependence suggests that softer ionization conditions can minimize cis/trans isomerization in the ion source. After separation using IMS, the cis and all-trans isomers of lycopene and β -carotene were shown by MS-MS with CID to produce unique fragmentation patterns that could be used to distinguish them. Until cis/trans isomerization of carotenoids can be eliminated in the ion source, the slow process of HPLC separation of geometrical isomers of carotenoids in biomedical samples will still be necessary for some applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Chemical structures of *cis*- and all-*trans*-lycopene and β -carotene.

Figure 2. Positive ion electrospray IM-MS drift time distributions of the M^+ ions of m/z 536.4 corresponding to *cis* (peak 1) and *all-trans* (peak 2) isomers of lycopene after infusion of (A) *cis/trans*-lycopene mixture (38/62); and (B) all-*trans*-lycopene. Note that isomerization occurred during electrospray so that the same *cis/trans* isomer ratio was observed for each lycopene sample.

Figure 3.

LC-IM-MS analysis of a mixture of β -carotene isomers using a C_{30} carotenoid HPLC column, positive ion electrospray and ion mobility separation prior to mass spectrometric detection. (A) LC-MS chromatogram showing β -carotene molecular ions of m/z 536.4 for each geometric isomer; (B) 2D map showing ion mobility drift time (y) vs. HPLC retention time (x). Ion mobility separation of molecular ions formed from 13-*cis*- β -carotene (retention time 7.2 min), 9-*cis*- β -carotene (retention time 9.9 min) and all-*trans*- β -carotene (retention time 8.8 min) indicated that all three species had isomerized in the ion source to form similar ratios of *cis*- β -carotenes (1-1, 1-2, 1-3) and all-*trans*- β -carotene (2-1, 2-2, 2-3).

Figure 4.

Effect of electro spray desolvation gas temperature on the abundances of cis (peak 1) and all-trans isomers (peak 2) of (A) lycopene; and (B) β -carotene following infusion of the all-trans isomer. Drift time spectra were acquired using positive ion electro spray mass spectrometry with detection of the molecular ions of m/z 536.4. (100 °C, dotted line; 200 °C, dashed line; 300 °C, solid line)

Figure 5. Relative abundances of cis isomers (peak 1 in Figure 2) and total (cis plus all-trans) isomers as a function of desolvation gas temperature for (A) lycopene; and (B) -carotene.

Figure 6. Positive ion electrospray IM-MS-MS CID spectra of the M^{+} ions of m/z 536.4 corresponding to (A) *cis*-lycopenes (ion mobility peak 1); and (B) all-*trans*-lycopene (ion mobility peak 2). t_d = drift time

Figure 7. Positive ion electrospray IM-MS-MS with CID of (A) *cis*-β-carotenes; and (B) all-*trans*-β-carotene. Note the relative abundances of m/z 444 and m/z 536 in the tandem mass spectra which distinguish the *cis* isomers from the all-*trans*-β-carotene. t_d = drift time

Table 1

Compound	IMS gas flow rate (mL/min)	Experimental CCSA ^a (±S.D.), Å ²	Theoretical CCS, Å ²
<i>Lycopene</i>	20	<i>cis</i> ^b	5- <i>cis</i>
		all- <i>trans</i> ^c	all- <i>trans</i>
	23	<i>cis</i> ^b	
		all- <i>trans</i> ^c	
<i>-Carotene</i>	20	<i>cis</i> ^b	13- <i>cis</i>
		all- <i>trans</i> ^c	all- <i>trans</i>
	23	<i>cis</i> ^b	
		all- <i>trans</i> ^c	

^aThe classical equation for CCS calculation was applied in Driftscope software as described by Williams *et al.*:²⁶

$$\Omega = [(18\pi)^{0.5}/16] [ze/(k_b T)^{0.5}] (1/m_{ion} + 1/m_{gas})^{0.5} (NK)^{-1}$$

z : the CCS of an analyte ion;
 z : the number of charges on the analyte ion;
 e : the charge on an electron;
 k_B : Boltzmann constant;
 T : temperature (K);
 m_{ion} : molecular mass of the analyte ion;
 m_{gas} : molecular mass of the IMS gas;
 N : the number density of the IMS gas;
 K : the mobility of analyte ion.
 b : First IM-MS peak, shorter drift time (mixture of cis isomers)
 c : Second IM-MS peak, longer drift time (all-trans isomer)