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Full Product Pattern Recognition in β-Carotene Thermal Degradation through Ionization Enhancement

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The full product pattern including both volatile and nonvolatile compounds was presented for the first time for β-Carotene thermal degradation at variable temperatures up to 600°C. Solvent-enhanced ionization was used to confirm and distinguish between the dissociation mechanisms that lead to even and odd number mass products.

Carotenoids as β-carotene are widely distributed in nature, where they play an important role in protecting cells and organisms. Their electronic delocalization is manifested in their optical properties and physical chemical reactivity with oxygenated radicals generated in photochemical reactions. Carotenes are known to be thermally unstable compounds.[1-6] A variety of degradation products has been identified, which led to debates on the degradation mechanisms among research groups using different solvent and analytical method at variable temperatures.[7-9] Previous mass spectrometry, including GC/MS and pyrolysis GC/MS were able to identify the low boiling fraction compounds, such as toluene, xylene, ionenes, and naphthalene. The high boiling fraction compounds had to be analyzed indirectly after the solvent washing of the residual nonvolatile materials. By hexane extraction, mass 444 and 378 were reported, which are correspondent to the elimination of toluene and dimethyl cyclodecapentaene from β-carotene, respectively.[3]

In this communication, we present the full product pattern of thermal degradation of β-carotene, dependent on both temperature and solvent molecules. Inside the apparatus of an atmospheric solid analysis probe mass spectrometer (ASAP-MS),[10,11] β-carotene crystals are attached at the end of a glass capillary which is within ~ 1 cm of the corona source and mass detector inlet. The hot nitrogen gas is then used to heat and bring the vaporized molecules immediately into the mass spectrometer after soft ionization at the ambient atmosphere, providing real-time, temperature dependent, highly sensitive mass analysis of the reaction process. The products from the radical dissociation processes are further evaluated through ionization enhancement. This method would be ultimately applicable to the characterization and evaluation of degradation or aging mechanisms of any non-volatile organic materials.

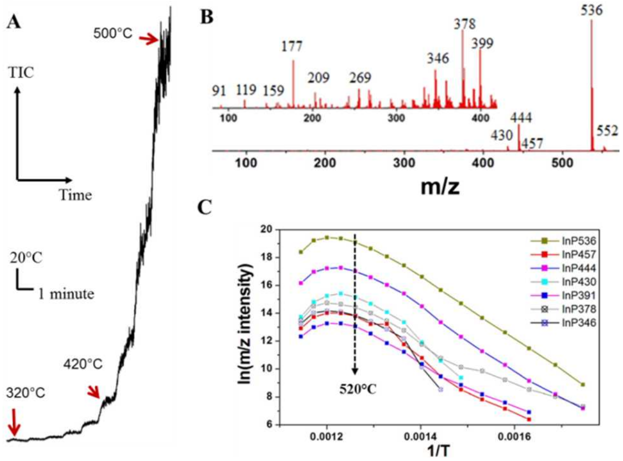

Fig. 1 Full product pattern in thermal degradation of β-carotene. (A) temperature dependent total ion chromatogram (TIC) versus time at 20°C increments per minute; (B) Mass spectrometric product pattern obtained at 500°C; (C) Arrhenius plots of the high mass number products in comparison to β-carotene evaporation (mass 536).

In the experiments, β-carotene was dispersed in acetonitrile and re-deposited at the end of a glass capillary. The temperature was increased in increments of 20°C per minute. At each step, the temperature increase was completed in less than 10 s then kept unchanged. As shown in Figure 1A, below 420°C the total ion chromatogram (TIC) increased slightly with the temperature and remained rather constant after the temperature variation. Above 420°C, TIC increased extensively with time, indicating rapid evaporation and decomposition of β-carotene. TIC then decreased at temperatures above 500°C and eventually returned to its background level when all the material had desorbed from the glass capillary (not shown in the figure). Because of the short mass

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Electronic Supplementary Information (ESI) available: [experimental, temperature dependent full product pattern spectra,and low energy collision fragmentation of mass 346 and mass 347]. See DOI: 10.1039/x0xx00000x diffusion length to the detector, each spectrum represents the real time chemical composition for each individual temperature. Simultaneously the high boiling fraction compounds were also detected with the low boiling fraction compounds, resulting in real time temperature-dependent full product patterns.

A representative spectrum averaged for 1 minute at 500°C is shown in Figure 1B. Mass 536 and 552 is assigned to β-carotene and its monoxide (536+16), respectively, confirmed as molecular ions from soft ionization. Among the products, the intensity of mass 444 is significantly larger than the other mass peaks. The other main products are shown by masses at 457, 430, 399, 378 and 346 with intense low number masses at 91, 105, 119, 159, 177, etc.

Mass 444 and 378 have been attributed to the loss of toluene (536-92) and dimethylcyclodecapentaene (536-158) from β-carotene, respectively.[3] Dimethylcyclodecapentaene is eventually dehydrogenated (-H_2) to dimethyl naphthalene. The reaction mechanism involves bond rearrangement of a β-carotene molecule into aromatic rings which are then eliminated.[3-5] The chemical structures of mass 444 and 378 are still similar to that of β-carotene but with shorter β-ring-to-ring distances. Products of mass 430 and 346 have not been reported previously. Mass 430 is potentially due to the elimination of xylene from β-carotene (536- 106). The identification of these two compounds is to be discussed in detail later.

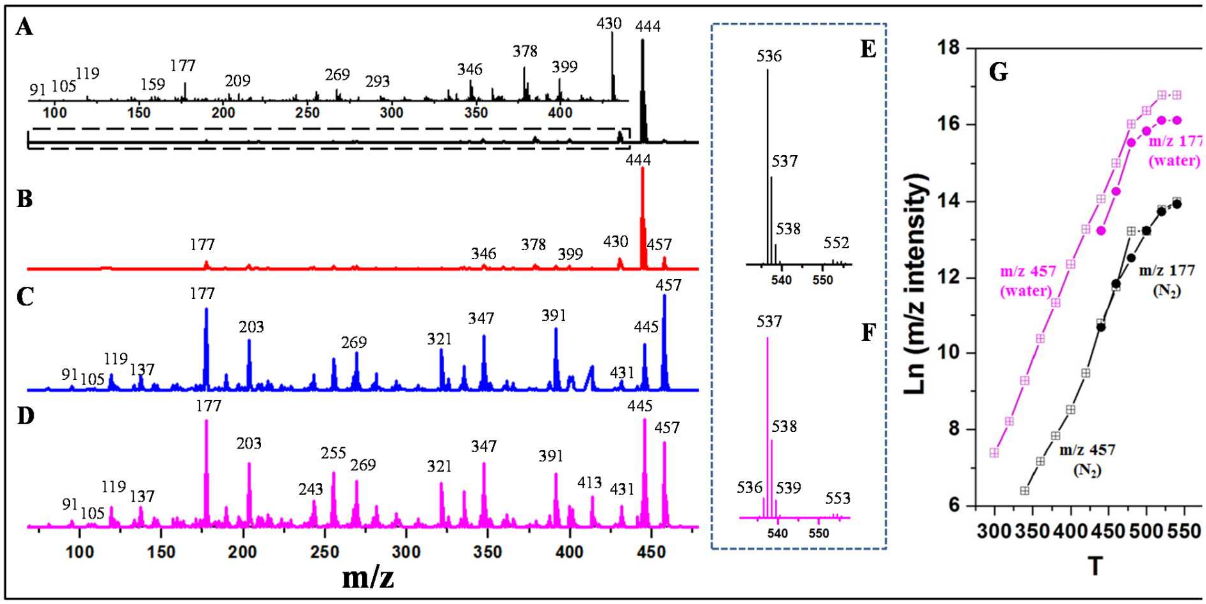

Fig.2 Solvent enhanced ionization. ASAP-MS (relative intensity) of β-carotene thermally degraded at 500 °C in (A, E) nitrogen, (B) nitrogen + dodecane, (C) nitrogen + methanol, and (D, F) nitrogen + water. (G) The ionization enhancement of odd number masses 457 and 177.

Masses 444 and 378/376 are generated at around 300°C when the spectrum starts to show β-carotene at mass 536. Further increasing the temperature, mass 430 appears, followed by mass 346 at the temperature around 450°C (Figure S1). Such temperature dependent product pattern variation indicates that those products are indeed due to thermal degradation rather than mass fragmentation.

Although the products appeared at different temperatures, the production rates are all exponential versus temperature, and with more or less similar slopes to that of β-carotene thermal evaporation. As shown in Figure 1C, only the high temperature products of mass 430 and 346 have slightly steeper slopes. A temperature transient is shown for mass 378. Note that masses 378 and 376 are generated simultaneously at temperatures ranging from 300 to 420°C. However, at the temperatures above 420°C, mass 378 becomes much more intense than mass 376, or mass 376 is diminished (Figure S1). Apart from mass 378, the other product ions show similar slopes in Arrhenius plots, suggesting they have similar activation energies and common mechanistic pathways of formation.

Above 420°C, mass peaks below 300 become apparent and intense. These mass peaks are all odd numbers, such as 91, 105, 119, 133, 159, 177, etc. As mentioned before, mass 159 is presumably assigned to the protonated dimethylcyclodecapentaene (158+1), and mass 177 to the protonated ionones (176+1). The ASAP-MS experiments with standard samples of toluene and xylene show mass 91 and 105, respectively. In addition to these low odd number masses, there are also high odd number masses. Products of masses at 457, 399, 391, and 359 appear at higher temperatures than the product of mass 444 (Figure 1C).

For hydrocarbons to be detected in the positive ion mode, the odd number masses have to be positively charged species, due to either (M-1) like toluene (92-1) and xylene (106-1) or (M+1) as dimethylcyclodecapentaene (158+1) and ionenes (176+1). Therefore, the odd number mass products are evident of radical degradation pathways through charge separation and bond scission.

To better address those odd number mass products, the ASAP-MS chamber was mixed with solvent vapour molecules.[9] Figure 2A and 2B show the product patterns obtained in the absence and presence of dodecane. Dodecane was chosen as an aprotic vapour similar to hexane[3] but with a much lower vapour pressure suitable for use in our high temperature experiment. The spectra patterns are similar to that discussed in Figure 1B. The even number masses at 536, 444, 430, and 378 are intense, while the odd number masses are relatively weak.

When the solvent was switched to methanol (Figure 2C) or water (Figure 2D), all even numbers are changed to odd numbers by +1. For example, β-carotene and its monoxide are changed from 536 and 552 to 537 and 553, respectively (Figure 2E, 2F). Note that a small fraction of mass 536 is still preserved in Figure 2F. All the odd number masses remain unchanged, but their intensities are significantly increased. Since the product patterns are almost identical in both cases, both water and methanol molecules are presumably not involved in the degradation reactions, apart from their contribution of protons.

The other noticeable differences after introducing water and methanol molecules include: (1) all the odd number masses are significantly enhanced in intensity (457, 391, 177, etc.) except mass 399; (2) only mass 346 is enhanced after protonation among all even number masses; (3) the relative intensity of mass 445 and 431 to 537 does not change; (4) mass 378 is diminished.

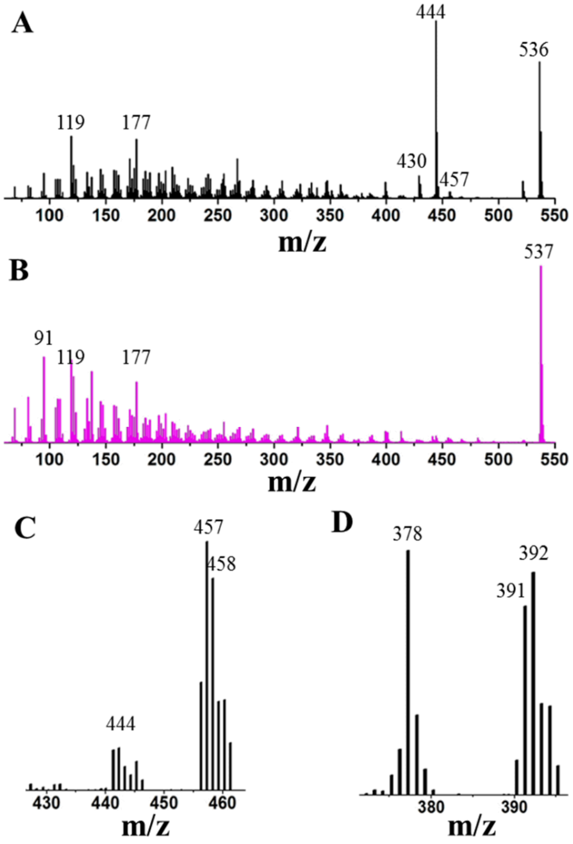

Fig. 3 Low energy collision fragmentation mass spectrometry. (A) β-carotene (mass 536) in N$_2$; (B) β-carotene (mass 537) and products of mass 457(C) and mass 391(D) in the presence of water vapour molecules; (C) and (D) illustrate the relationship between the odd and even number masses. The collision energy: 20 eV.

Figure 2G plotted the ionization enhancement for masses 457 and 177 impacted by water molecules. The enhancement factors seem unchanged with temperature, showing from 10 to 20 times enhancement. In both cases, mass 177 starts to appear at 440°C, while mass 457 appears ~40°C lower in the presence of water molecules.

Why does the ionization enhancement selectively occur on the odd number masses? Figure 3 and 4 provide insights. In the presence of collision energy, neutral β-carotene (mass 536) shows significantly intense even number fragments of masses 444 and 430 (Figure 3A), and only at high collision energies (i.e., higher than 40 eV) do the even number masses (444, 430, 378, and 346) disappear. At low collision energies, protonated β-carotene (mass 537) does not show high mass number fragments above 300 (Figure 3B). Instead, fragmenting protonated β-carotene leads to primarily odd number masses that are below 300. With high collision energies, both neutral and protonated β-carotene are all fragmented into odd number masses at 91, 105, 119, and 177. The results illustrate two dissociation mechanisms of which the radical process is favoured for the protonated β-carotene at low and high collision energies but only for high collision energies for neutral β-carotene.

Figure 3C and 3D demonstrate the relationships between the odd and even number masses. Mass 457 shows the fragment of mass 444, while mass 391 is fragmented to mass 378. It is also interesting to note that multiple masses are shown for the odd number mass products and their fragments. Besides the isotopic mass distribution, these multiple masses (e.g., 390, 391, 392, 393, 394, and 395) illustrate the potential processes involved for the radical species, such as (de)protonation (±1) and (de)hydrogenation (-H$_2$) or single to double bond transitions (±2).

Under electron impact with increased collision energies, the mass 458 is still weaker than mass 457 (Figure 3C), while mass 392 is more intense than mass 391 (Figure 3D). Figure S2 also compares mass 346 to mass 347 at collision energy of 30 eV. Mass 347 is more favourable in both cases. Such dependence of relative mass intensity on collision energy reveals the selectivity in protonation. The product of mass 346 is easily protonated and therefore is more abundant in protic solvents and at high collision energies. Figure 2 shows that the product of mass 378 diminishes in the presence of protic solvents, except when high collision energies are applied (Figure 4D). This suggests that in protic solvents mass 391/392 is the more favoured products while mass 378 only forms under these conditions due to fragmentation of mass 391. In contrast, under aprotic conditions mass 378 is the favoured product.

The intensities of high odd number masses (457, 391, and 347) are enhanced by water and methanol molecules because they are produced from radical dissociation processes. In light of the relationship between odd and even number mass products and the clusters of multiple masses (e.g., 456, 457, 458, 459, and 460), we assume that the aromatic rearrangement is preserved under protic conditions. However, in the breakdown processes, one methyl group is favoured to stay with the main chain or high molecule weight product, resulting in product of mass 458 instead of 444 and mass 392 instead of 378, respectively. Such a transition or bond delocalization occurs preferentially for protonated β-carotene.

The steric distribution of β-carotene and some high mass number degradation products is illustrated in the ion mobility mass spectrometry shown in Figure 4. Protonated β-carotene (m/z 537) shows shorter drift times than the neutral one (m/z 536), all with multiple peaks. The shorter the drift time, the higher the mobility.[12,13] The large difference in their steric distribution indicates that the protonated β-carotene is much more folded while neutral β-carotene stays in its elongated conformation (longer drift time and lower mobility). The products of mass 457, 445, 430 and 391 all show multiple broad distributions and therefore multiple conformations. Mass 347 shows only a single narrow peak, indicating that the two β-rings may be broken apart and therefore any conformation variation does not cause a significant separation based on ion mobility.

We can therefore propose the possible degradation processes for β-carotene. As shown in Figure 4, eliminating toluene leads to mass 445/444 (red), and xylene to mass 431/430 (green). Both products still possess two β-rings and multiple conformations. Direct breakdown of β-carotene at 9-10 and 9’-10’ double bonds leads to elimination of ionene and mass 347/346 (pink). The elimination of dimethylcyclodecapentaene to mass 378 is not favoured from protonated β-carotene. Instead, the product of mass 391/392 becomes apparent (orange). Together with mass 457, these odd number mass products are formed when one methyl group remains in the main chain after the radical dissociation and elimination of aromatic compounds. Trace oxygen in the ionization chamber oxidizes β-carotene, but the observed product patterns in the experimental temperature range are not due to oxidative cleavage.[14, 15]

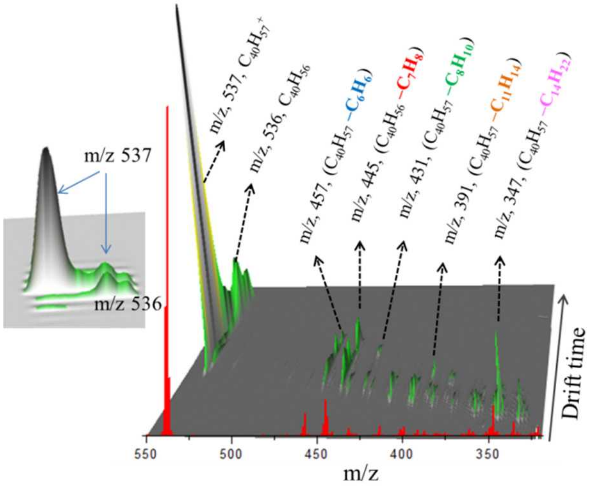

Fig. 4 Drift-time ion mobility mass spectrometry and proposed thermal degradation mechanism. Temperature: 450°C. Solvent vapour: water.

In summary, based on atmospheric solid analysis probe mass spectrometry, the full product pattern of thermal degradation of β-carotene has been observed and evaluated. Both volatile and non-volatile chemical compounds are simultaneously detected, and the product pattern is temperature dependent. Oxygen and solvent molecules are not readily involved in the decomposition processes.
In addition to mass 444 and 378 and masses for toluene, xylene, and dimethyl naphthalene, several more products are observed in real-time in this work. Mass 430 is believed to be due to the elimination of xylene. Mass 346 appears at relatively higher temperatures, and its intensity is significantly enhanced by the water and methanol molecules. It is assumed to be due to a direct breakdown of 9-10 and 9’-10’ double bonds from the charged β-carotene radicals, probably due to the elimination of ionenes. The full product pattern modulated by the solvent molecules confirms that the radical dissociation processes that lead to odd number mass products are significantly enhanced by the protic solvent molecules.

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