Kinetics and Mechanism of the Primary Steps of Degradation of Carotenoids by Acid in Homogeneous Solution

Alan Mortensen* and Leif H. Skibsted

Food Chemistry, Department of Dairy and Food Science, Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

The kinetics of reaction between trifluoroacetic acid as an acid of medium strength and the carotenoids β-carotene, zeaxanthin, canthaxanthin, and astaxanthin has been examined in detail including the effects of dioxygen, acid concentration, and carotenoid structure. Reaction between acid and carotenoid leads to species absorbing in the red and near-infrared (NIR) spectral regions, intermediates that subsequently disappear. ESR experiments clearly show that these species are not carotenoid radicals, although their NIR absorption is similar to the absorption of carotenoid radical cations. Under most reaction conditions, the disappearance of carotenoids follows pseudo-zero-order kinetics, whereas the reaction order is >1 with respect to acid, and the long-lived (hours) intermediates are suggested to be mono- (700 nm) and diprotonated carotenoid (~950 nm). Acid induces *cis*/*trans*-isomerization via the protonated intermediates, which also decay to nonradical species with shorter conjugated systems—most probably carotenoid esters. Slow protonization of the methine carbon is the primary step in the degradation, but dioxygen increases the rate as a result of formation of a charge-transfer complex with the carotenoids as indicated by a red-shift of the NIR absorption bands. Carotenoids with carbonyl groups (astaxanthin and canthaxanthin) have slower rates of degradation than β-carotene and zeaxanthin, indicating preferential nondegradative protonation of the carbonyl groups.

Keywords: *β-Carotene; zeaxanthin; canthaxanthin; astaxanthin; acid degradation*

INTRODUCTION

Carotenoids are strongly colored (yellow to red) pigments and scavengers in plants of reactive excited species such as triplet chlorophyll and singlet dioxygen. Carotenoids may also function as primary antioxidants in biological systems by scavenging peroxyl radicals (Mortensen and Skibsted, 1998). Due to their extended conjugated system, carotenoids are susceptible to attack by a variety of reactive chemical agents. Of main interest in recent years has been the reaction with radicals in relation to antioxidative action. Carotenoids react with different types of radicals by different mechanisms. Carotenoids thus scavenge nitrogen dioxide by forming the one-electron oxidized species, the carotenoid radical cation, exclusively, whereas scavenging of thiyl–sulfonyl radicals leads to formation of the carotenoid radical cation and a precursor of the carotenoid radical cation (Everett et al., 1996; Mortensen et al., 1997). On the other hand, scavenging of thiyl radicals does not form carotenoid radical cations; rather, an adduct between the thiyl radical and the carotenoid is formed (Everett et al., 1996; Mortensen et al., 1997), and phenoxyl radicals are scavenged by parallel formation of two intermediate species, one of which is a carotenoid radical cation (Mortensen and Skibsted, 1996, 1997). Carotenoids are believed to function as antioxidants due to the formation of such resonance-stabilized radical cations or radical adducts, which are not capable of participating in autoxidation reactions. The preferred mode of antioxidant action of carotenoids seems to be by electron transfer rather than transfer of hydrogen atoms. Carotenoid radical cations are characterized by their strong absorption around 1000 nm and a rather long lifetime compared to many other radicals (in the millisecond regime); both absorption maximum and lifetime depend strongly on solvent.

Besides attack by radicals, carotenoids are also degraded by light (Jørgensen and Skibsted, 1990; Mortensen and Skibsted, 1999; Pesek and Warthesen, 1987), heat (Henry et al., 1998; Kanasawud and Crouzet, 1990; Sharma and Le Maguer, 1996), and acid (Chen et al., 1995; Tekale and Joshi, 1977). In all three degradation mechanisms, dioxygen plays a major role. Carotenoids may be degraded to form *cis*/*trans*-isomers affected by any of the above-mentioned three factors, or they may be oxidized to form new compounds usually giving rise to loss of color and in some cases formation of aroma compounds.

Carotenoids have been found to be protonated by nitric acid and moderately strong acids such as trichloroacetic acid and trifluoroacetic acid. The protonated form of the carotenoid has been shown to absorb strongly (Wassermann, 1954, 1957, 1959a,b; Buchwald and Jencks, 1968; Mallik et al., 1978, 1980; Ioffe et al., 1976; Jeevarajan et al., 1994) in the same spectral region as the carotenoid radical cation (Edge et al., 1997), that is, ~1000 nm. Carotenoids also react with Lewis acids such as iodine to give a species absorbing in this spectral region (Malik et al., 1978, 1980; Ioffe et al., 1976). Acid is known to initiate degradation of carotenoids in food and beverages by a largely unknown mechanism (Chen et al., 1995; Tekale and Joshi, 1977).

* Author to whom correspondence should be addressed (telephone +45 35 28 32 90; fax +45 35 28 33 44; e-mail am@kvl.dk).

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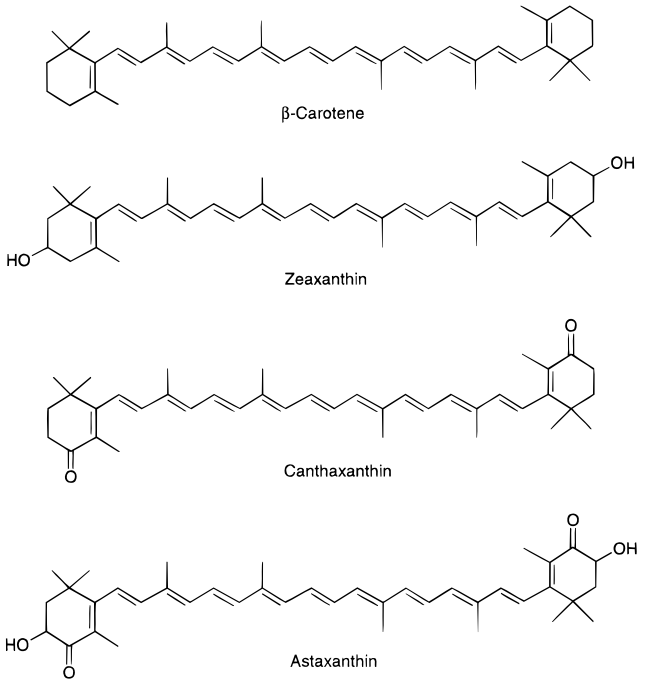

Figure 1. Structures of the four carotenoids.

In the previous studies, protonation of carotenoids, including the effects of carotenoid structure, presence of dioxygen, time, and concentration of acid, was not studied in any detail, which has prompted us to further investigate the protonation of carotenoids as the initial step in nonoxidative degradation of carotenoids.

EXPERIMENTAL PROCEDURES

The carotenoids β-carotene, zeaxanthin, astaxanthin, and canthaxanthin sealed in ampules under argon were supplied by Roche A/S. Trifluoroacetic acid (Uvasol), 1,4-dioxan (pro analysi), triethylamine (for synthesis), and benzene (pro analysi) from Merck were used as received. Fresh stock solutions of carotenoid in benzene were prepared for each experiment.

In experiments without dioxygen, dioxygen was removed from a solution of carotenoid in benzene by three freeze−pump−thaw cycles, after which trifluoroacetic acid was distilled in vacuo to this frozen solution, which was subsequently thawed. UV−vis absorption measurements began right after the solution had thawed completely. In all experiments, with or without dioxygen, the volume of trifluoroacetic acid added to a solution of carotenoid in benzene was ≤1% of the total volume.

UV−vis spectra were measured with an HP 8453 diode array spectrophotometer from Hewlett-Packard using quartz cells with a path length of 1 cm (Hellma). The samples were thermostated at 20 ± 0.5 °C. The time course of reaction was studied by measuring spectra every 15 min. ESR experiments were performed on a JEOL JES−FR 30 ESR spectrometer employing X-band radiation for excitation.

RESULTS

The kinetics of the reactions of four carotenoids, in which all combinations of the presence or absence of hydroxyl and carbonyl groups in the 3,3′ and 4,4′ positions are present (Figure 1), with trifluoroacetic acid as an example of a medium strength acid, was studied in homogeneous solution by following changes in the UV−vis absorption as a function of acid concentration and the presence or absence of dioxygen. β-Carotene, a carotene, was studied in most detail. The reactions of the xanthophylls zeaxanthin, canthaxanthin, and astaxanthin are compared to the reactions of β-carotene to assess the influence of the two functional groups.

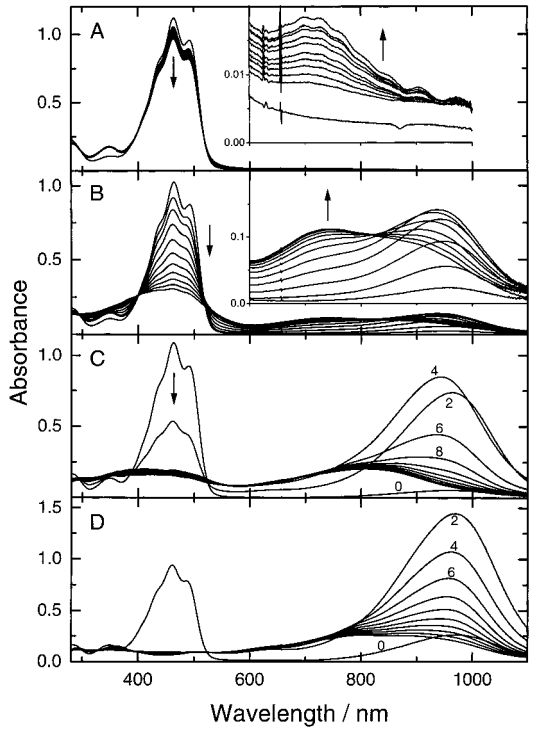

Figure 2. Absorption spectra of 10 μM β-carotene in aerated benzene after addition of trifluoroacetic acid to a concentration of 0.01 (A), 0.05 (B), 0.10 (C), and 0.50 M (D). The spectra are in intervals of 2 h. Arrows indicate the change in absorption as a function of time. Numbers on the spectra indicate time (in hours) after addition of trifluoroacetic acid.

***β*-Carotene.** In Figure 2 are shown the spectra of aerated solutions of β-carotene with various concentrations of trifluoroacetic acid. Trifluoroacetic acid causes the characteristic absorption spectrum of β-carotene with a maximum at 464 nm to gradually disappear. At the same time new absorptions in the red and near-infrared (NIR) spectral region appear. A relatively high concentration (~0.01 M) of acid is needed to bring about reaction to an appreciable extent (Figure 2A), and increasing the acid concentration, besides accelerating the degradation of β-carotene, also has a profound affect on the NIR absorption. At the lowest acid concentration an absorption band around 700 nm (Figure 3A) with a number of distinct maxima appears gradually but has not reached its maximum absorption after 20 h of reaction. The shape of this band does not change as a function of time, indicating that it is due to one species or due to a number of closely related species, that is, *cis−trans* isomers (see below), following similar kinetics for their formation. A new band around 350 nm also appears (Figure 2), and the β-carotene absorption band is slightly blue-shifted (1−2 nm), both observations indicating the formation of *cis*-β-carotenes (Britton, 1995). Higher concentrations of acid lead to the formation of new absorption bands. At a trifluoroacetic acid concentration of 0.05 M a band around 950 nm is observed (Figure 2B). This band seems to gradually shift toward shorter wavelengths as a function of time (Figure 3B). The band around 700 nm is still present, although its distinct features (Figure 2A) are masked by the other absorption band. Increasing the acid concentration further still shifts the NIR absorption

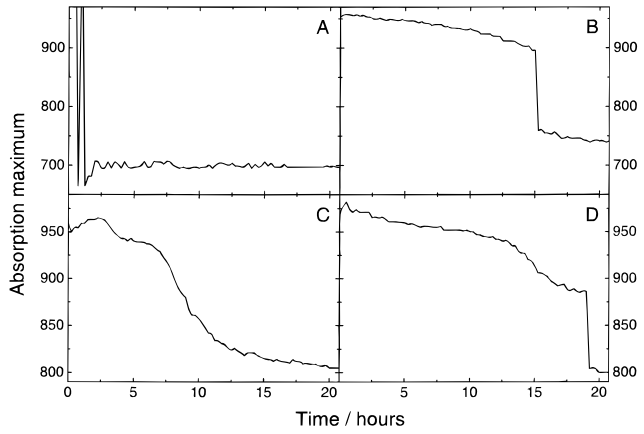

Figure 3. Change in absorption maximum in the region 650−1100 nm as a function of time in solutions of 10 μM β-carotene in aerated benzene with 0.01 (A), 0.05 (B), 0.10 (C), and 0.50 M (D) trifluoroacetic acid.

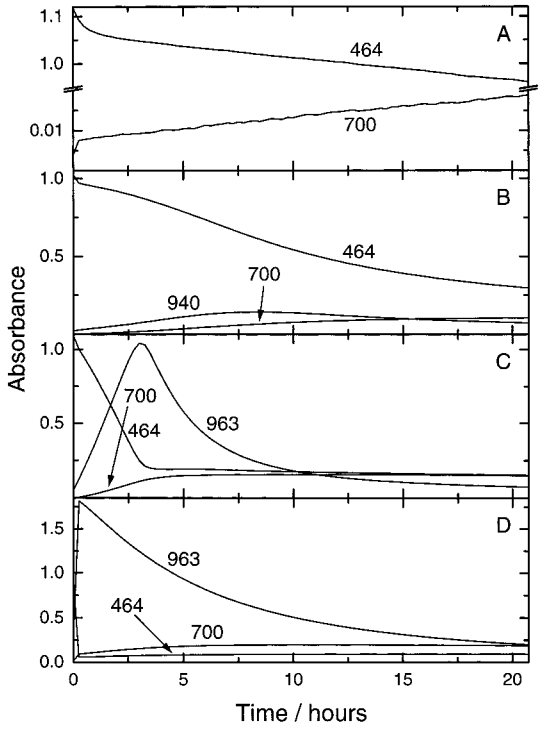

Figure 4. Time traces of absorption changes of 10 μM β-carotene in aerated benzene after addition of trifluoroacetic acid to a concentration of 0.01 (A), 0.05 (B), 0.10 (C), and 0.50 M (D). Numbers on the traces indicate the wavelength in nanometers.

band slightly toward longer wavelengths (Figure 3C,D) and the intensity of the band increases (Figure 2C,D). The absorption band around 700 nm is probably still there as an increased absorption from 520 nm and upward is observed.

The reaction between β-carotene and trifluoroacetic acid is not instantaneous, and after 20 h, reaction is still taking place (Figure 2). The concentration of the acid not only determines which species are being formed but also influences the rate of reaction (Figures 2 and 4). At an acid concentration of 0.01 M, after 3 h of reaction, the disappearance of β-carotene seems to follow zero-order kinetics with a rate constant of 1.2×10^{-11} M s^{-1} (Figure 4A). Formation of the species absorbing at 700 nm also follows zero-order kinetics. The kinetics seems to be more complicated at an acid concentration of 0.05

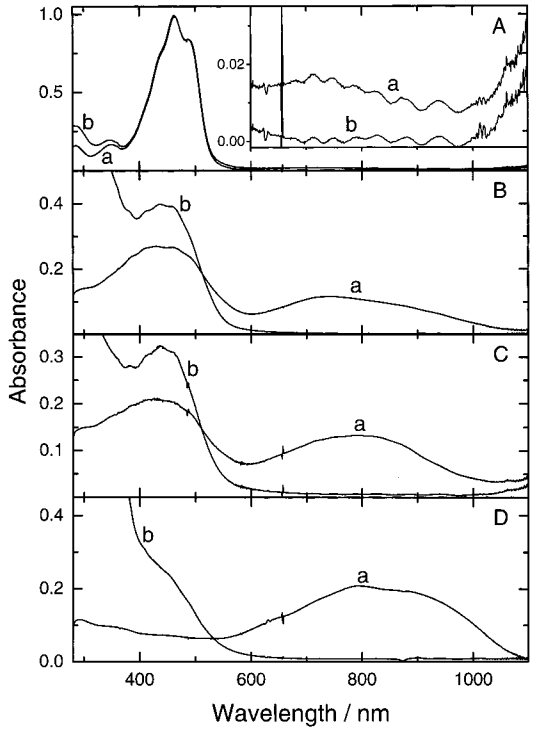

Figure 5. Absorption spectra of 10 μM β-carotene in aerated benzene 21 h after addition of trifluoroacetic acid to a concentration of 0.01 (A), 0.05 (B), 0.10 (C), and 0.50 M (D): (a) before addition of triethylamine; (b) after addition of triethylamine. Triethylamine has a strong absorption band at 300 nm showing its foot in the blue-green spectral region.

M (Figure 4B); that is, there is no apparent simple decay of β-carotene. Increasing the acid concentration to 0.10 M again seems to lead to zero-order kinetics both for the disappearance of β-carotene and for the buildup of the NIR-absorbing species (Figure 4C). The rate constant of disappearance of β-carotene is 7.1×10^{-10} M s^{-1}. At the highest concentration of acid, the reaction is too fast to determine its order with the present type of experiments, but if it follows zero-order kinetics, the rate constant is $>8.8 \times 10^{-9}$ M s^{-1}.

Neutralization of the acidic solutions with dioxan or triethylamine resulted in disappearance of the red and near-infrared absorption and partial recovery of absorption between 400 and 500 nm (Figure 5). However, addition of base did not fully regenerate β-carotene, and the longer a solution of β-carotene and trifluoroacetic acid at a given concentration had been allowed to react, the smaller the extent of recovery. After 21 h of reaction, addition of base still led to an increase in absorption around 400−500 nm. However, the absorption maximum after the addition of triethylamine to the solutions containing 0.05 and 0.10 M trifluoroacetic acid is at 436 nm compared to 464 nm for β-carotene.

The spectra of solutions of β-carotene and trifluoroacetic acid in the absence of dioxygen (Figure 6) are different from the spectra of aerated solutions (Figure 2). The absence of dioxygen first of all leads to slower degradation of β-carotene compared to aerated solutions with the same concentration of trifluoroacetic acid. Second, the absorption maximum is found at 910 nm (Figure 6), whereas in aerated solutions it is found at 950−975 nm (Figure 3), and the absorption maximum at time zero does not seem to depend on trifluoroacetic acid concentration as it does in the case of aerated solutions (Figure 3). At a concentration of 0.5 M tri-

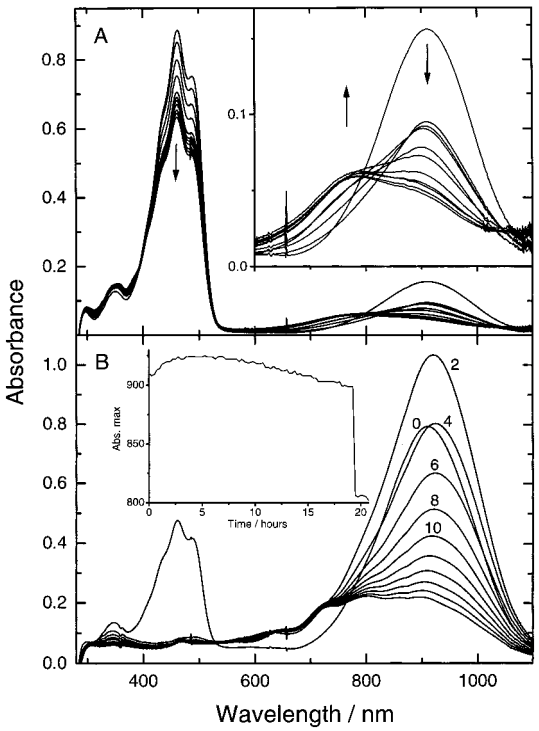

Figure 6. Absorption spectra of 10 μM β-carotene in benzene after addition of trifluoroacetic acid to a concentration of 0.1 (A) and 0.5 M (B). The solutions were degassed by three freeze−pump−thaw cycles (see Experimental Procedures). The spectra are in intervals of 2 h. Arrows indicate the change in absorption as a function of time. Numbers on the spectra indicate time (in hours) after addition of trifluoroacetic acid. The insert in (B) shows the change in absorption maximum in the region 650−1100 nm as a function of time.

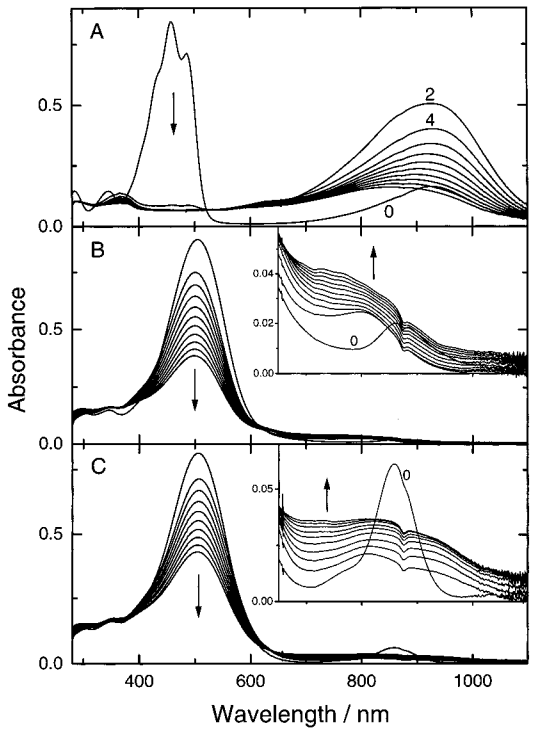

Figure 7. Absorption spectra of 10 μM zeaxanthin (A), canthaxanthin (B), and astaxanthin (C) in aerated benzene after addition of trifluoroacetic acid to a concentration of 0.50 M. The spectra are in intervals of 2 h. Arrows indicate the change in absorption as a function of time. Numbers on the spectra indicate time (in hours) after addition of trifluoroacetic acid.

fluoroacetic acid the absorption maximum shifts toward higher wavelength with time (from 910 to 925 nm, Figure 6B, insert), a behavior not observed with the aerated solutions (Figure 3). Third, in solutions containing 0.1 M trifluoroacetic acid the strongest NIR absorption is encountered after 3 h in an aerated solution (Figure 4C), whereas in a deaerated solution maximum absorption occurs at time zero, that is, right after thawing. Again, the so-called *cis*-band is observed at 350 nm together with a slight blue-shift of the β-carotene absorption band (Figure 6). The effect of acid concentration on reaction rate seems to be even more pronounced in deaerated solutions than in aerated solutions. The rate of degradation of β-carotene in a deaerated solution containing 0.1 M trifluoroacetic acid is thus similar to that of aerated solutions containing between 0.01 and 0.05 M trifluoroacetic acid (Figures 2A,B and 6A), whereas at a trifluoracetic acid concentration of 0.5 M the rate of β-carotene degradation is only slightly slower in a deaerated solution than in its aerated counterpart (Figures 2D and 6B).

Addition of triethylamine to deaerated solutions of β-carotene and trifluoroacetic acid also leads to partial recovery of absorption around 400−500 nm. In that respect, a deaerated solution containing 0.1 M trifluoroacetic acid resembles an aerated solution containing 0.01 M trifluoroacetic acid (Figure 5A), and the deaerated solution with 0.5 M trifluoroacetic acid resembles the aerated solution of the same concentration (Figure 5D).

Solutions of trifluoroacetic acid and β-carotene were ESR-silent during both formation and decay of the NIR absorption, showing that radicals are not products or intermediates at any significant concentration.

Xanthophylls. The reaction between zeaxanthin and trifluoroacetic acid seems to follow the same trend as the reaction between β-carotene and trifluoroacetic acid, except that zeaxanthin reacts somewhat more slowly (Figure 7A). A *cis*-band at 345 nm is observed, the zeaxanthin absorption band is blue-shifted by a few nanometers, and the NIR absorption band is also blue-shifted to 930 nm. The influence of dioxygen is qualitatively the same as in the case of β-carotene; that is, the absorption maximum in a deaerated solution with 0.5 M trifluoroacetic acid is at 855 nm at time zero and shifts to 910 nm after ~2 h before decreasing to lower wavelengths.

The two xanthophylls containing carbonyl groups react in the same way with trifluoroacetic acid. Reaction with trifluoroacetic acid primarily leads to a red-shift and broadening of the absorption bands at 483 nm (canthaxanthin) and 488 nm (astaxanthin). At a trifluoroacetic acid concentration of 0.5 M the absorption maximum of these two absorption bands is at 506 nm (Figure 7B,C) and at 500 nm in the presence of 0.1 M trifluoroacetic acid. These two xanthophylls are degraded much more slowly by trifluoroacetic acid than β-carotene and zeaxanthin (Figures 2D and 7A), and a high concentration of acid (>0.1 M) was needed to get an appreciable extent of reaction. Addition of trifluoroacetic acid to solutions of these two carotenoids gives rise to an absorption band at 860 nm, which "rapidly" decays (within an hour) giving way to a broad absorption without a distinct maximum (inserts in Figure 7B,C). Whether dioxygen influences the reaction is difficult to assess as only a broad featureless absorption

above 650 nm is observed. The rate of degradation of the xanthophylls does not seem to be influenced much; this is comparable to β-carotene, for which it was found that at high acid concentrations (0.5 M), the rate of degradation was only slightly slower in a deaerated solution than in an aerated solution.

DISCUSSION

Reaction between carotenoids and trifluoroacetic acid yields species absorbing in the NIR spectral region (Figures 2, 6, and 7). The carotenoid radical cation formed by reaction with a number of different radicals also absorbs strongly in the same spectral region (Edge et al., 1997). Trifluoroacetic acid is able to oxidize a number of aromatic compounds to yield radical cations, a reaction that is accelerated by light (Eberson and Radner, 1992). Whether a thermal electron transfer reaction is possible or not is less clear, but if it does take place, it is very slow (Eberson and Radner, 1992). The solutions in the present experiments were kept in the dark of the cell holder and exposed to the analyzing light for only a short period every 15 min. We did not observe any signal in the ESR spectra of solutions of carotenoids and trifluoroacetic acid, clearly demonstrating that the NIR absorption, due to species at micromolar concentrations, in this case is not due to the carotenoid radical cation. Furthermore, addition of base to solutions of carotenoid and trifluoroacetic acid regenerated the carotenoid if addition was shortly after addition of acid, which would not have been possible if the carotenoid had been oxidized by the acid.

Simple conjugated alkenes have been shown to be protonated by acid to form carbocations sufficiently long-lived for their UV spectra to be obtained. The absorption maximum is red-shifted compared to the parent compound: the absorption maximum of allylic carbocations (formed by protonation of conjugated dienes) is at ∼300 nm, whereas pentadienyl carbocations (formed by protonation of conjugated trienes) absorb at 400 nm (Deno et al., 1962, 1963; Deno and Pittman, 1964).

Carotenoid Structure. The introduction of hydroxy groups as in zeaxanthin decreases the reactivity of the carotenoid toward trifluoroacetic acid slightly as evidenced by a slower rate of reaction. However, the reactions seem to be the same as is observed with β-carotene (Figures 2 and 7A). Xanthophylls with carbonyl groups (astaxanthin and canthaxanthin) react much more slowly with triflouroacetic acid (Figure 7B,C), giving rise to only weak absorption in the NIR. Whereas the carotenoid absorption bands of β-carotene and zeaxanthin are blue-shifted by a few nanometers due to formation of *cis*-isomers (see below), the absorption bands of canthaxanthin and astaxanthin are gradually red-shifted as the concentration of trifluoroacetic acid increases because these two carotenoids are mainly protonated on their carbonyl groups (Buchwald and Jencks, 1968). This reaction, however, seems to be rapid compared to the reaction leading to species absorbing in the NIR. The slightly slower rate of reaction of zeaxanthin compared to that of β-carotene could be due to the hydroxy groups, which compete with the conjugated double bonds for the proton. In the case of astaxanthin and canthaxanthin the carbonyl groups are part of the conjugated system, whereas the hydroxy groups of zeaxanthin are not, and protonation of these is hence not expected to modify the protonation of the conjugated system drastically.

The discussion in the following will mainly focus on β-carotene but will also pertain to zeaxanthin and in part to canthaxanthin and astaxanthin.

Kinetics. Proton transfer to and from nitrogen or oxygen is usually a diffusion-controlled process in the thermodynamically favorable direction (Crooks, 1977). However, proton transfer reactions involving carbon are usually slower than this and may indeed be far from diffusion-controlled, even for thermodynamically favorable reactions (Hibbert, 1977). One reason for this is that hydrocarbons, such as β-carotene, do not easily form hydrogen bonds with acids (Hibbert, 1977). Simple alkenes have been shown to react slowly with trifluoroacetic acid: the first-order rate constants for 1-hexene [in this case the data were not accurate enough to determine the order of the reaction (Peterson, 1960)] and 1-octene (Mason and Norman, 1973) in trifluoroacetic acid at 25 °C are 5.1×10^{-5} and 1.2×10^{-5} s^{-1}, respectively.

Although reaction between carotenoids and trifluoroacetic acid is slow and this by itself is not surprising (see above), the kinetics and the spectra indicate that there is more to the reaction than a simple acid−base equilibrium. First of all, the reaction between carotenoids and trifluoroacetic acid is of higher order than 1 in trifluoroacetic acid (Figure 4). At low concentrations of acid (0.01 M) one species with an absorption maximum around 700 nm (Figure 2A) is formed, and higher concentrations lead to the formation of a new absorption around 950 nm (Figure 2B−D). This and the observed dependence of the rate of reaction on trifluoroacetic acid concentration can be explained by two concurrent reactions

$$\mathrm{Car} + \mathrm{CF_3COOH} \rightleftarrows [\mathrm{CarH^+}\cdots\mathrm{CF_3COO^-}] \quad (1)$$

$$\mathrm{Car} + 2\mathrm{CF_3COOH} \rightleftarrows [\mathrm{CarHH^{2+}}\cdots 2\mathrm{CF_3COO^-}] \quad (2)$$

where the species at 700 nm is the monoprotonated carotenoid and the species absorbing around 950 nm is the dication. Most likely, ion pairs and not free ions are formed in benzene as a solvent. In most of the previous studies, high concentrations of acid (∼1 M) were used and only the species absorbing around 950 nm was observed (Wasserman, 1954, 1957, 1959a,b; Buchwald and Jencks, 1968; Ioffe et al., 1976). However, in one study it was found that an intermediate was formed upon dissolution of β-carotene (735 nm) or β-apo-8′-carotenal (632 nm) in dichloromethane (Jeevarajan et al., 1994). This species, possibly a charge-transfer complex (Jeevarajan et al., 1994), was formed by reaction with small amounts (0.1−0.2 mM) of HCl naturally occurring in dichloromethane. Addition of HCl_{aq} to a concentration of 1.0 M to a solution of β-carotene, β-apo-8′-carotenal, or canthaxanthin (871 nm) resulted in NIR absorption believed to be due to the carotenoid radical cations (Jeevarajan et al., 1994). This assignment was solely based on the NIR absorption, and in our view it is more likely that protonated species are formed as we observed no ESR signal in our experiments. Reactions 1 and 2 would give the following rate equation (disregarding the reverse reactions)

$$-\mathrm{d[Car]}/\mathrm{d}t = (k_1[\mathrm{CF_3COOH}] + k_2[\mathrm{CF_3COOH}]^2)[\mathrm{Car}] \quad (3)$$

which is pseudo-first-order when $[\mathrm{CF_3COOH}] \gg [\mathrm{Car}]$,

as in our experiments. However, in many cases the order seems to be zero (Figure 4A,C,D), indicating that the reaction is more complicated than indicated by reactions 1 and 2.

Two possibilities arise: (i) the kinetics are for some reason truly pseudo-zero-order or (ii) the reaction seems to be pseudo-zero-order, for instance, due to formation of other species absorping in the same spectral region. That the reaction between carotenoids and trifluoroacetic acid is not fully described by reactions 1 and 2 is, however, evident. First, the products of reactions 1 and 2 are not stable but undergo further reactions (Figure 2). One of these is isomerization evidenced by absorption around 350 nm

$$\textit{trans}\text{-Car} + CF_3COOH \rightleftarrows [\textit{trans}\text{-CarH}^+\cdots CF_3COO^-] \quad (1)$$

$$[\textit{trans}\text{-CarH}^+\cdots CF_3COO^-] \rightleftarrows [\textit{cis}\text{-CarH}^+\cdots CF_3COO^-] \quad (4)$$

$$\textit{trans}\text{-Car} + [\textit{cis}\text{-CarH}^+\cdots CF_3COO^-] \rightleftarrows [\textit{trans}\text{-CarH}^+\cdots CF_3COO^-] + \textit{cis}\text{-Car} \quad (5)$$

$$[\textit{cis}\text{-CarH}^+\cdots CF_3COO^-] \rightleftarrows \textit{cis}\text{-Car} + CF_3COOH \quad (6)$$

which has been shown to take place in dichloromethane as well (Jeevarajan et al., 1994). In reaction 5 proton transfer between two carotenoid molecules takes place followed by formation of a new ion pair. Whereas protonation of carotenoid is a slow process (Figure 2A), equilibrium between *cis*- and *trans*-isomers is attained in a couple of hours, as evidenced by the development of the *cis*-band and the gradual loss of structure of the carotenoid absorption band due to formation of *cis*-isomers having absorption bands that are slightly blue-shifted (Britton, 1995). This can also explain why the absorption at 464 nm decreases more rapidly during the first 2−3 h of reaction (Figure 4A), because the absorption of the *cis*-isomers is weaker at this wavelength; whereas after equilibrium has been attained, all isomers decay at the same rate (the linear portion after 2−3 h), that is, they react with trifluoroacetic acid in the same manner and with a rate comparable to that of the *trans*-isomer. This can also explain the gradual shift of the maximum absorption to shorter wavelength (Figure 3) because the *cis*-isomers formed "rapidly" in reaction 6 are slowly protonated and their absorption, by analogy to the parent compounds, is likely to be blue-shifted compared to the protonated *trans*-isomer. Second, the scheme represented by reactions 1 and 2 does not take into account the presence of intermediates. The reaction is more adequately described by the scheme (Hibbert, 1977)

$$\text{Car} + CF_3COOH \rightleftarrows [\text{Car}\cdots CF_3COOH] \rightleftarrows [\text{CarH}^+\cdots CF_3COO^-] \quad (7)$$

where [Car···CF_3COOH] is a hydrogen-bonded complex. Formation of this complex is usually slow (Hibbert, 1977) (the equilibrium lies to the left or the far right). The rate equations for these reactions are

$$-\text{d[Car]}/\text{d}t = k_a[\text{Car}][CF_3COOH] - k_{-a}[\text{Car}\cdots CF_3COOH] \quad (8)$$

$$\text{d[Car}\cdots CF_3COOH]/\text{d}t = k_a[\text{Car}][CF_3COOH] + k_{-b}[\text{CarH}^+\cdots CF_3COO^-] - (k_{-a} + k_b)[\text{Car}\cdots CF_3COOH] \quad (9)$$

$$\text{d[CarH}^+\cdots CF_3COO^-]/\text{d}t = k_b[\text{Car}\cdots CF_3COOH] - k_{-b}[\text{CarH}^+\cdots CF_3COO^-] \quad (10)$$

If it is assumed that k_{-a} and k_b are much higher than k_a and k_{-b}, that is, formation of the hydrogen-bonded complex is rate-determining (Hibbert, 1977), the steady-state approximation can be applied to eq 9, d[Car···CF_3COOH]/dt = 0, and inserted in eq 8 to give

$$-\text{d[Car]}/\text{d}t = (k_a - k_a k_{-a}/(k_{-a} + k_b))[\text{Car}][CF_3COOH] - k_{-a}k_{-b}/(k_{-a} + k_b)[\text{CarH}^+\cdots CF_3COO^-] \quad (8a)$$

If $k_b \ll k_{-a}$, that is, the equilibrium lies to the left in eq 7, which is not unreasonable considering that a high concentration of trifluoroacetic acid is nessecary to protonate β-carotene, this simplifies to

$$-\text{d[Car]}/\text{d}t = -k_{-b}[\text{CarH}^+\cdots CF_3COO^-] \quad (8b)$$

If the assumption $k_b \gg k_{-b}$ (see above) is applied to eq 10

$$\text{d[CarH}^+\cdots CF_3COO^-]/\text{d}t = k_b[\text{Car}\cdots CF_3COOH] \quad (10a)$$

it can be seen that formation of protonated carotenoid is pseudo-zero-order ([Car···CF_3COOH] is constant according to the steady-state approximation) and so is degradation of carotenoid (eq 8b).

Protonation of 2,3,6,7-tetramethoxy-9,10-dimethylanthracene by trifluoroacetic acid (Eberson and Radner, 1992) likewise seems to proceed via (pseudo) zero-order kinetics, and pseudo-zero-order kinetics may thus be a general feature of protonation of a number of unsaturated systems.

Decay of Protonated Carotenoids. The protonated carotenoids decay slowly to unidentified species absorbing at shorter wavelengths (Figures 2 and 3). These species are also protonated, and the absorption band of these species after deprotonation is blue-shifted compared to the absorption band of the carotenoid (Figure 5). This all points to the formation of species with shorter conjugated systems. It thus seems likely that trifluoroacetic acid adds to the double bonds of the carotenoids to give carotenyl trifluoroacetates similar to the products formed by reaction with 1-hexene and 1-octene (Peterson, 1960; Mason and Norman, 1973). However, in the case of carotenoids more than one trifluoroacetic acid may add to the double bonds, which also seems to be what the kinetics imply.

Usually, carbocations are short-lived, but in the case of carotenoids with their extended conjugated system, the positive charge is stabilized by delocalization and the reaction between trifluoroacetate and protonated carotenoid to give an ester is hence slow.

Influence of Dioxygen. In the reactions above, dioxygen is not involved in the reactions between trifluoroacetic acid and carotenoids. Dioxygen does not readily react with carbocations as both are electrophilic. However, it is evident that dioxygen has a profound effect on the rate of reaction and on the NIR absorption

(Figures 2 and 6). It can only be speculated what the mechanism is, but it may involve addition of dioxygen to the protonated carotenoid followed by rearrangement and deprotonation to yield, for example, endoperoxides or hydroperoxides. This would shift the equilibrium in reaction 7 to increase the rate of reaction.

Carotenoids in juices (carrot, tomato, orange, etc.) are exposed to a slightly acidic environment. Acidification of carrot juice with citric acid leads to a decrease in the content of carotenoids (α- and β-carotene and lutein) and perhaps a slight increase in the content of *cis*-isomers (Chen et al., 1995). The carotene and xanthophyll contents of lucerne juice decreased significantly (up to 60%) during storage for 24 h (Tekale and Joshi, 1977). However, at high pH (10 and 8) the rate of degradation of carotenoid was significantly shorter than at lower pH (6.5 and 5.4). Although the conditions in a juice cannot be directly compared to the conditions in our experiments, it seems that the acidity of juices leads to increased degradation of carotenoids, which should be considered together with the other degradation mechanisms of carotenoids (heat, light, etc.).

Conclusions. Trifluoroacetic acid, although capable of oxidizing certain aromatic compounds, does not generate carotenoid radicals, as evidenced by the lack of ESR signals, but rather protonates the carotenoids.

Carotenoids are protonated slowly by medium strength acids such as trifluoroacetic acid. The reaction order with respect to acid is higher than 1 and probably involves monoprotonation (low concentration of acid) and diprotonation (high concentration of acid), whereas the reaction order with respect to carotenoid is pseudo-zero-order. *cis*/*trans*-Isomerization is induced by protonation, and the reaction products are probably carotenoid esters. Carotenoids containing carbonyl groups are preferentially protonated on this group and not on a carbon atom of the conjugated system, whereas hydroxy groups alter the reactivity of the carotenoid only slightly. This explains the higher stability of carotenoids with carbonyl groups compared to that of other xanthophylls and carotenes. Dioxygen also modified the reactions leading to a higher rate and absorption bands in the NIR which are red-shifted compared to the absorption band of deaerated solutions.

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