Synthetic Food Color: Photosensitized Decarboxylation of Peptides

The light-induced reactions between synthetic food colors and peptides has been investigated by spin trapping and electron spin resonance spectroscopy. It was found that the two certified food color additives of the triphenylmethane type, FD&C Blue No. 1 and FD&C Green No. 3, were able to photosensitize the decarboxylation of di- and tripeptides when the mixture was irradiated with light that was exclusively absorbed by the dye. The decarboxylation radicals were spin trapped by 2-methyl-2-nitrosopropane (MNP) and identified by electron spin resonance (ESR). The other four synthetic dyes tested, FD&C Red No. 3, FD&C Red No. 40, FD&C Yellow No. 5, and FD&C Yellow No. 6, were not able to sensitize this reaction. A mechanism based on an electron transfer from the carboxyl end of the peptide to the photoexcited dye is suggested for this reaction.

In spite of the extensive, and maybe excessive, use of food color additives in processed food, only scarce reports on photochemical reactions induced by these compounds and visible light on other food components have been published (Rosenthal, 1983). Chan (1975) examined nine artificial food colors as potential photosensitizers for the oxidation of fatty acids and found that only FD&C Red No. 3 could initiate that process. We recently found that FD&C Blue No. 1, FD&C Green No. 3, FD&C Red No. 4, FD&C Yellow No. 5, and FD&C Yellow No. 6 generated hydroxyl radicals when photoexcited in aqueous solutions. The generation of hydroxyl radicals was explained by an electron transfer process from water to the electronically excited pigment (Carmichael et al., 1983).

We describe here further studies on reactions photochemically induced by synthetic food colors in the presence of peptides in aqueous solutions, as a step in clarifying the interaction between light and food components. These reactions have been studied by spin trapping and electron spin resonance (ESR). In recent years, the method of spin trapping has been developed for stabilizing transient free radicals. The method consists of the chemical addition of the short-lived free radical to an unsaturated double bond to yield a new and stable free radical at room temperature (Lagercrantz, 1971; Janzen, 1980). The spin trap 2-methyl-2-nitrosopropane (MNP) has been used extensively for trapping and identifying free radicals derived from amino acids and pyrimidine bases (Riesz and Rosenthal, 1982). The pertinent chemical reaction for this process is

$$R\cdot + (t\text{-Bu})N{=}O \rightarrow R(t\text{-Bu})N{-}\dot{O} \leftrightarrow R(t\text{-Bu})\dot{N}^{+}{-}O^{-}$$

Following this procedure, radicals can be detected at ambient temperatures without suppressing thermally activated processes and solvation interactions with the medium.

It was the aim of this study to identify the primary transients generated in peptides, as a model for proteins, by the combined action of light and synthetic pigments.

MATERIALS AND METHODS

Chemicals. Peptides were purchased from Sigma Chemical Co. and were used without further purification. 2-Methyl-2-nitrosopropane (MNP) was obtained from Aldrich Chemical Co. Food colors were acquired from commercial sources and were used without further purification. Aqueous MNP solutions (1 mg/mL) were prepared by stirring overnight in the dark. The concentration of the peptides in MNP solutions was 0.1 M and that of the pigment ranged from 10^{-6} to 10^{-3} M.

Irradiation. The photolyses were performed in situ at room temperature in aerated solutions, in the standard aqueous quartz cell (60 × 10 × 0.25 mm) placed in the ESR cavity, using a Schoeffel 1000-W high-pressure Hg–Xe lamp coupled with a Schoeffel grating monochromator. The excitation light had a maximum centered at 313 ± 10 nm.

ESR Measurements. A Varian E-9, X-band spectrometer was used for ESR measurements. The magnetic field modulation frequency was 100 kHz. The scans were traced with a modulation amplitude equal to or lower than 0.2 G, and the microwave power level was maintained at 10 mW to avoid saturation. The samples were exposed to irradiation during scanning.

RESULTS AND DISCUSSION

Aliphatic amino acids and peptides hardly absorb any light above 220 nm; aromatic amino acids such as phenylalanine, tyrosine, and tryptophan possess only a marginal absorption above 300 nm. Consequently, this class of compounds is hardly affected by the range of wavelengths in the solar light at ground level or by artificial light.

We found that the irradiation of aliphatic di- and tripeptides with light of λ = 313 nm in the presence of two certified food color additives, FD&C Blue No. 1 (Brilliant Blue FCF CI No. 42090) and FD&C Green No. 3 (Fast Green FCF CI No. 42053), efficiently generate free radicals that could be easily trapped by MNP and characterized.

The ESR spectrum of the nitroxide spin adduct shows a primary triplet due to the nitrogen atom of the nitroxide function as well as secondary splittings that arise from the magnetic nuclei of the trapped radical. These secondary splittings allow the tentative identification of the trapped radical. A 17.2-G triplet due to the di-*tert*-butyl nitroxide radical was generated as an undesirable side product.

Thus the illumination of glycylglycine in the presence of food colors and MNP produced the spectrum shown in Figure 1. The large, primary nitrogen triplet (a_N = 16.2 G) displayed additional splittings consisting of 3 × 3 lines due to a secondary nitrogen (a_N^{β} = 2.70 G) and two equivalent hydrogens (a_H^{β} = 9.9 G). This easily recognizable pattern is consistent with that of the decarboxylated glycylglycine residue.

An identical reaction pattern was obtained for the few other peptides tested. Thus glycylalanine yielded a spectrum composed of 3 × 4 lines due to the primary nitrogen as well as the equivalent secondary β-nitrogen and β-proton (a_N = 16.0 G, a_N^{β} = 2.18 G, and a_H^{β} = 2.18 G) as expected for the spin adduct of the decarboxylation radical of glycylalanine (Figure 2). Similarly, glycylvaline generated a spectrum composed of 3 × 3 × 2 lines with a_N = 15.8 G, a_N^{β} = 2.45 G, and a_H^{β} = 1.0 G (Figure 3).

All these spectra are in agreement with the signals previously reported for the decarboxylation of the same peptides by ultraviolet photolyses at λ = 220 nm (Lion et al., 1980).

In this study, we have also tested the isolated amino acids, glycine and alanine, under similar reaction condi-

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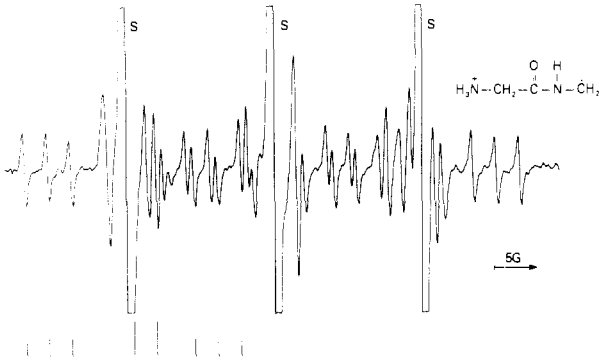

Figure 1. ESR spectrum of an irradiated aqueous solution containing Blue No. 1, glycylglycine, and MNP. The structure shown is that of the untrapped radical. S denotes di-*tert*-butyl nitroxide.

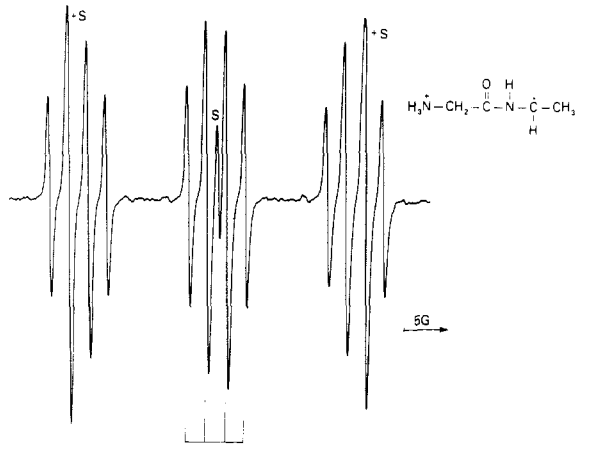

Figure 2. ESR spectrum of an irradiated aqueous solution containing Blue No. 1, glycylalanine, and MNP. The structure shown is that of the untrapped radical. S denotes di-*tert*-butyl nitroxide.

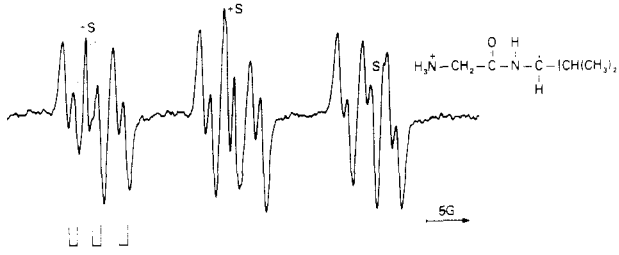

Figure 3. ESR spectrum of an irradiated aqueous solution containing Blue No. 1, glycylvaline, and MNP. The structure shown is that of the untrapped radical. S denotes di-*tert*-butyl nitroxide.

tions. However, none of them yielded stable, well-defined radicals. Conversely, the tripeptides glycylglycylalanine, alanylglycylglycine, and alanylalanylalanine yielded the corresponding decarboxylation radicals whose spectra are similar to those observed for the dipeptides.

Control experiments performed by irradiating mixtures of MNP and a peptide in the absence of dyes with light of λ = 313 nm generated only di-*tert*-butyl nitroxide.

Four other certified food colors were tested for their capability of inducing this photochemical reaction: FD&C Red No. 40 (Allura Red AC CI No. 16035), FD&C Red No. 3 (Erythrosine CI No. 45430), FD&C Yellow No. 5 (Tartrazine CI No. 19140), and FD&C Yellow No. 6 (Sunset Yellow FCF CI No. 15985). However, none of them photogenerated recordable radicals under our experimental conditions. It appears that the common feature of the photoactive colors is the triphenylmethane structure.

The formation of the decarboxylation radical can be explained by an electron transfer from the carboxyl group to the excited dye. The resulting carboxyl radical loses CO_2 to yield the observed paramagnetic species.

This suggested mechanism must be reconciled with our previous observation that the irradiation of synthetic food colors in aqueous solutions generates hydroxyl radical as we showed by spin trapping with 5,5-dimethyl-1-pyrroline 1-oxide (Carmichael et al., 1983). The reaction of hydroxyl radical with dipeptides is known to generate radicals found by hydrogen atom abstraction (Joshi et al., 1978) that were not detected in the present reaction with peptides. Consequently, it appears that the electron transfer process between a peptide and a dye is preceded by the formation of a short-lived exciplex.

Since photosensitized decarboxylation reactions are observed in solutions of dye with concentrations comparable to those used in food systems ([dye] $\geq 10^{-6}$ M), it is possible that these reactions could occur in foods during prolonged exposure to lower intensity light (e.g., fluorescent). Therefore, it is obvious that if the photoinduced decarboxylation of peptides by food color additives were also to occur in an actual food system, it would lead to nutritional degradation due to the chemical modification of proteins. This possibility is being investigated.

Registry No. FD&C Blue No. 1, 3844-45-9; FD&C Green No. 3, 2353-45-9; glycylglycine, 556-50-3; glycylalanine, 3695-73-6; glycylvaline, 1963-21-9; glycylglycylalanine, 19729-30-7; alanylglycylglycine, 3146-40-5; alanylalanylalanine, 5874-90-8.

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