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Phenolic Components of Brown Scales of Onion Bulbs Produce Hydrogen Peroxide by Autooxidation

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The outer scales of onion bulbs turn brown during maturation. Molecular oxygen was reduced to H_2O_2 when the brown scales were suspended in water. Brown components isolated from the brown scales also transformed molecular oxygen into H_2O_2. During the autooxidation process, absorbance in the visible region was increased. On acid hydrolysis of the brown fraction, 2, 4, 6-trihydroxyphenylglyoxylic acid, 3, 4-dihydroxybenzoic acid and the quinone form of benzoic acid were detected. In addition, glucose was detected as a sugar. 3, 4-Dihydroxybenzoic acid was preferentially oxidized during autooxidation of the brown fraction. One of the oxidation products was the quinone form. Stable electron spin resonance (ESR) signals were detected in the brown fraction. New ESR signals appeared on oxidation of the brown fraction by hexacyanoferrate (III). One of the newly formed radicals seemed to have a 3, 4-dihydroxyphenyl group. Based on these results, possible structures, mechanism of H_2O_2 formation and biological significance of the brown components are discussed.

Key words: Antimicrobial agent — Browning — ESR signal — Hydrogen peroxide — Onion (*Allium cepa*) bulbs — Quinhydrone structures

The presence of large amounts of quercetin glucosides in yellow onion bulbs has been reported (Bilyk *et al.* 1984, Kiviranta *et al.* 1988, Hirota *et al.* 1998, Tsushida and Suzuki 1996), whereas no quercetin glucosides were detected in white onion bulbs (Tsushida and Suzuki 1996). The glucosides are mainly localized in the abaxial epidermis of the scales and the levels are higher in the outer than in the inner scales (Walker and Stahman 1955, Hirota *et al.* 1999). The outer scales of yellow onion bulbs turn brown on maturation, whereas those of white onion bulbs do not. Yellow bulbs are more resistant to infection than white onion bulbs (Walker and Stahman 1955). The difference in the sensitivity between the two kind bulbs has been discussed in relation to the presence or absence of an antifungal agent, 3, 4-dihydroxybenzoic acid (DHBA) (Friend 1979, Walker and Stahman 1955). In drying onion scales, DHBA is formed by oxidation of quercetin, which is enhanced by peroxidase (Takahama and Hirota 2000). Quercetin is formed by deglucosidation of the glucosides (quercetin 4′-glucoside and quercetin 3, 4′-diglucoside). In addition to DHBA, 2, 4, 6-trihydroxyphenylglyoxylic acid is also formed by oxidative degradation of quercetin in the process of browning (Takahama and Hirota 2000).

In general, phenolics are autooxidizable and during the autooxidation of phenolics, O_2^- and H_2O_2 are formed (Bors *et al.* 1997). Polymers of phenolics such as lignins (Oniki and Takahama 1997) and melanins (Korytowski *et al.* 1985, Oniki and Takahama 1992) can also be oxidized by molecular oxygen. During oxidation, O_2^- and H_2O_2 are formed (Korytowski *et al.* 1985). Brown components, which are formed from chlorogenic acid in the apoplast of tobacco leaves on aging, also reduce molecular oxygen to H_2O_2 (Takahama *et al.* 1999). If brown components of mature onion scales, which may be oxidation products of quercetin, can be oxidized by molecular oxygen, H_2O_2 may be formed. The H_2O_2 thus formed can function as an antimicrobial agent (Mehdy *et al.* 1996). In this paper, we deal with formation of H_2O_2 by brown components of the brown scales and with some characteristics of the components that participate in the formation of H_2O_2.

Materials and Methods

Plant materials

Onion bulbs (*Allium cepa* L. cv. kamui) were obtained from Takii (Kyoto). Outer dried brown scales were used for experiments.

Fractionation

Dried brown scales (50 g) were broken into small pieces, then extracted with 5 liters of water for 12 hr at about 10 C. The extract was dried with a rotary evaporator at about 35 C (yield, 3.10 g dry weight). The residue was dissolved in 50 ml of water. After centrifugation (3,000×*g*, 5 min), 350 ml of methanol was added to the supernatant. The reddish gelatinous precipitate was removed by centrifugation. The methanol solution obtained was dried with a rotary evaporator. These procedures were repeated three times. The

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Abbreviations: DHBA, 3, 4-dihydroxybenzoic acid; ESR, electron spin resonance

residue obtained after the above procedures was dissolved in 50 ml of water. This water solution (pH, about 4.5) was extracted successively with ether (50 ml), ethyl acetate (50 ml) and butanol (10 ml). Extraction by each solvent was repeated three times. After evaporation of solvents, the residue of each fraction was weighed. The yields of ether extract, ethyl acetate extract, butanol extract and remaining water fraction were 0.36, 0.23, 0.35 and 1.02 g, respectively. The water-soluble fraction obtained after organic solvent extraction was used to prepare a fraction to examine consumption of molecular oxygen and to measure electron spin resonance (ESR) signals. Butanol extract was used to isolate a yellow component referred to as P440 in this study.

Preparation of brown fraction

The water-soluble fraction prepared as described above (0.2 g) was dissolved in 1 ml of 50% methanol and applied to a Sephadex LH-20 (Pharmacia Biotech, Uppsala, Sweden) column (2.5 cm i.d.×40 cm). After washing the column with methanol (1.5 ml min^{-1}) to remove yellow and red components that migrated as bands, yellowish brown components were eluted with 500 ml of 50% methanol at a rate of 1.5 ml min^{-1}. All the eluent was collected and dried with a rotary evaporator at about 35 C. The yield was 44 mg. This component was referred to as the brown fraction.

Isolation of P440

The butanol extract (0.1 g) prepared as described above was dissolved in 20 ml of water and pH of the solution was adjusted to 2 with 2.5 M HCl. This acidic solution was extracted with 20 ml of ethyl acetate, and then 10 ml of butanol. The butanol extract was dried with a rotary evaporator and the residue was dissolved in 2 ml of 50% methanol for application to a cellulose (CF11, Whatman, Maidstone, UK) column (2 cm i.d.×20 cm). A yellow band, which was eluted with 50% methanol (1.5 ml min^{-1}), was collected. This yellow substance was chromatographed again using the cellulose column and 50% methanol. The yellow substance obtained after the second chromatography step was purified further by Sephadex LH-20 column chromatography (2.5 cm i.d.×40 cm) using methanol as an eluent (1.5 ml min^{-1}). The yield was about 1 mg and the product was referred to as P440. The P440 was reduced by a small amount of sodium borohydride in 0.1 M NaH_2PO_4 bubbled with N_2 gas. The reduction product was extracted with ethyl acetate three times. The extract was combined and dried with a rotary evaporator. After dissolving in methanol, the reduction product was identified by HPLC (see below).

Phenolics and sugars of the brown fraction

The brown fraction (1 mg) was dissolved in 0.3 ml of water, then 0.3 ml of 2.5 M HCl was added. The acidic solution was incubated for 20 min at about 95 C. After cooling, the solution was extracted with 1 ml of ethyl acetate three times, then 1 ml of butanol, because not all P440 was extracted with ethyl acetate. The ethyl acetate and butanol extracts were combined and the solvents were evaporated with a rotary evaporator. The residue was dissolved in 0.3 ml of methanol to analyze phenolics by HPLC (see below). The remaining acidic solution was incubated further. After incubation for 3 hr at about 95 C, the acidic solution was dried with a rotary evaporator (about 35 C) to analyze sugars by cellulose thin-layer chromatography (0.1 mm thickness, Merk, Darmstadt, Germany). The solvents for the chromatography were phenol saturated with water and a mixture of butanol, toluene, pyridine and water (5 : 1 : 3 : 3, v/v) (Harborne 1984). Sugars were developed by spraying the mixture of aniline (0.92 ml) and phthalic acid (1.6 g) dissolved in 100 ml of butanol saturated with water.

To discuss the stability of phenolics of the brown fraction, 7 mg of the brown fraction was dissolved in 7 ml of 0.1 M sodium phosphate (pH 7.0) and incubated at about 15 C. After incubation for desired periods, 0.5 ml of the solution was withdrawn and 0.5 ml of 2.5 M HCl was added. The acidic solution was heated for 20 min at about 95 C. After cooling, the acidic solution was extracted successively with ethyl acetate and butanol as described the above. The ethyl acetate and butanol extracts were combined to determine the levels of phenolics by HPLC.

HPLC analysis

HPLC was performed using a Shim-pack CLC-ODS column (6 mm i.d.×15 cm) (Shimadzu, Kyoto). 2, 4, 6-Trihydroxyphenylglyoxylic acid, DHBA and P440 were detected and identified using a spectrophotometric detector with a photodiode array (Shimadzu M 10A) with a mixture of methanol and 25 mM KH_2PO_4 (1 : 4, v/v) as the mobile phase. Their levels were estimated from areas under the peaks on chromatograms at 260 nm for the phenolic acids and 440 nm for P440.

Oxygen uptake

Oxygen uptake was measured using an oxygen electrode obtained from Rank Brothers (Cambridge, UK). When oxygen uptake of dried brown scales was measured, the reaction mixture (2 ml) was composed of 20 mg of dried brown scales in 0.1 M sodium phosphate (pH 6.0). Oxygen uptake of the water extract of dried brown scales was measured in the reaction mixture (2 ml) that contained 1 ml of the extract (1 g dry weight of brown scales per 50 ml of water) and 1 ml of 0.1 M sodium phosphate (pH 6-8). Oxygen consumption by the brown fraction was measured in a reaction mixture (1 ml) that contained 0.1 ml of 3 mg ml^{-1} of the brown fraction and 0.9 ml of 0.1 M sodium phosphate (pH 6-8). The brown fraction was dissolved in water. The pH of the solution was about pH 4.5.

Spectrophotometric measurements

UV and visible absorption spectra of the brown fraction were measured in a reaction mixture (1 ml) that contained 0.03 mg of the brown fraction in 0.1 M sodium phosphate (pH 7.0) at about 15 C using a Hitachi 557 spectrophotometer (Tokyo). Changes in the absorption spectra during autooxidation and by addition of sodium borohydride, cysteine and mercaptoethanol were also measured in the above mixture.

ESR spectra

ESR spectra were measured using a JEOL JES-FE1XG spectrometer at about 15 C with a quartz flat cell (0.05 ml) under the following conditions: microwave power, 1 mW; scanning speed, 0.625 mT min^{-1}. Other conditions for measurements are described in the Figure legends. The reaction mixture was prepared under an argon atmosphere and the mixture contained 9.2 or 12 mg ml^{-1} of the brown fraction in 0.2 M KCl-0.2 M NaOH buffer (pH 13) (McKenzie 1969). Potassium hexacyanoferrate (III) was added under anaerobic conditions.

Reagents

Catalase was from Boehringer Mannheim GmbH (Germany). DHBA and 3, 4-dihydroxybenzaldehyde were obtained from Wako Pure Chem. Ind. (Osaka). 2, 4, 6-Trihydroxyphenylglyoxylic acid was synthesized according to the method reported by Hargreaves *et al.* (1958).

Results

Formation of H_2O_2

When dried brown scales were suspended in buffer solution, molecular oxygen was consumed (Fig. 1, trace A-1). During oxygen consumption, the reaction mixture turned a reddish brown color indicating that brown components were dissolved in the buffer solution. On addition of catalase (1,300 unit) to the reaction mixture, oxygen was evolved indicating the formation of H_2O_2 during oxygen consumption. From this trace, it was calculated that about 30 μM H_2O_2 was formed during consumption of about 55 μM O_2. No oxygen evolved following the second addition of catalase or when catalase was added to the buffer solution (data not shown).

Water extracts of dried brown scales also absorbed molecular oxygen (Fig. 1, trace A-2). The rate of oxygen uptake was higher at high pH than at low pH in the range from pH 6 to 8. Furthermore, the brown fraction prepared as described in Materials and Methods also consumed molecular oxygen, and the rate increased with increases in pH value (traces B-1, B-2 and B-3). On addition of catalase to the reaction mixtures, oxygen was evolved. Again, about half of the consumed oxygen was transformed to H_2O_2.

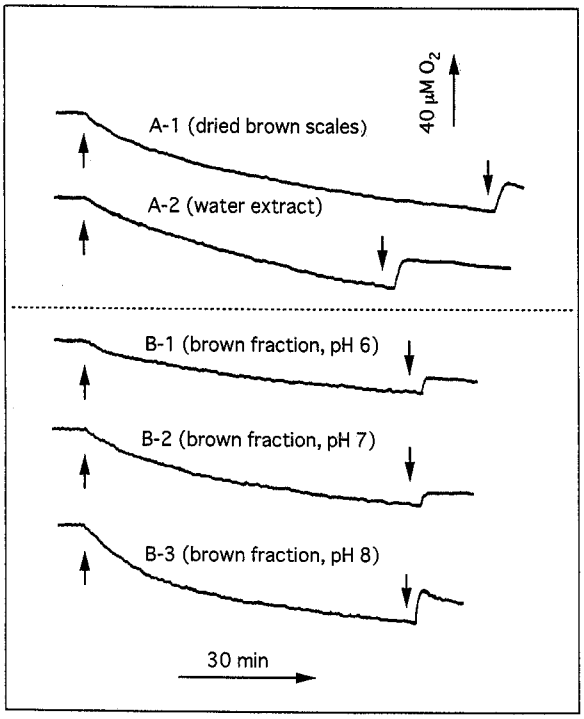

Fig. 1. Time courses of oxygen uptake. Reaction mixtures for the measurements are shown in Materials and Methods. Trace A-1, dried brown scales; trace A-2, water extract of dried brown scales. Traces from B-1 to B-3: Brown fraction. B-1, pH 6; B-2, pH 7; B-3, pH 8. Upward arrows, addition of samples; downward arrows, addition of catalase (1,300 unit).

Spectrophotometric measurements

The absorption spectrum of the brown fraction had peaks at about 295, 330 and 490 nm at pH 7 (Fig. 2, lower panel, trace 1). During incubation of the reaction mixture, absorbances at 270, 370 (shoulder) and 495 nm increased (upper

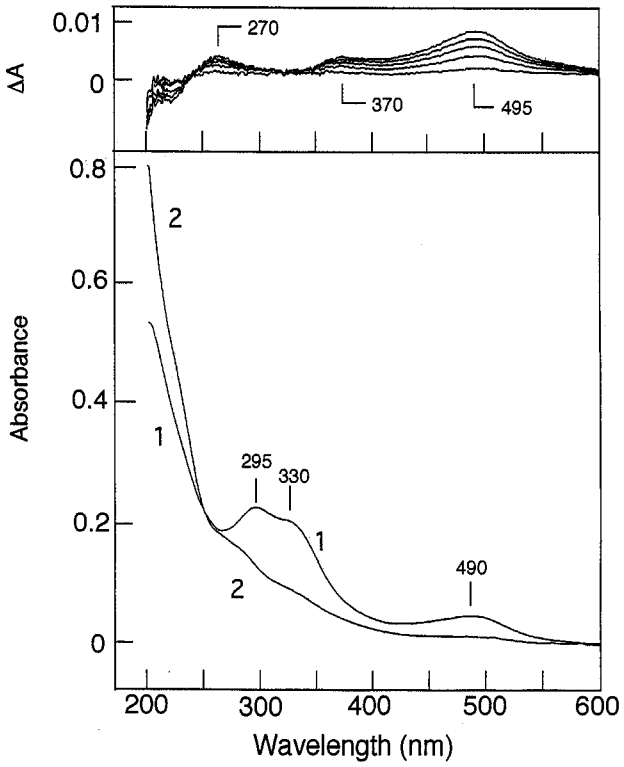

Fig. 2. Redox reactions of the brown fraction. Upper panel: Difference spectra measured during autooxidation of the brown fraction. The absorption spectrum (curve 1) was recorded as a reference, and difference spectra were recorded after 0, 10, 20, 30 and 50 min. Lower panel: Absorption spectrum of the brown fraction and reduction products. Curve 1, brown fraction; curve 2, sodium borohydride reduced. The reaction mixture (1 ml) contained 0.03 mg of the brown fraction in 0.1 M sodium phosphate (pH 7.0). Scanning speed was 120 nm min^{-1} and light path length was 4 mm.

panel). Such changes in absorption spectra were also observed at pH 6 and 8. the rate of increase in absorbance became faster as pH was increased from 6 to 8, suggesting that the increase may be due to autooxidation of phenolics contained in the brown fraction. The absorption bands of the brown fraction at 295, 330 and 490 nm were decreased and absorption in the wavelength range below 250 nm was increased when a small amount of sodium borohydride was added with bubbling N_2 gas. The absorption band in the visible region was decreased with addition of mercaptoethanol and cysteine (1-100 mM), but not with addition of ascorbic acid (1-100 mM) (data not shown). The results shown in Fig. 2 indicated that the brown fraction consisted of oxidizable and reducible components.

Phenolic components

When water solutions of the isolated brown fraction were directly analyzed by HPLC, trace amounts of several components were detected as peaks (Fig. 3, panel A). In addition, components, which had absorption bands in UV and visible regions, were also eluted gradually. Absorption spectra of the eluted components were essentially the same regardless of the retention time. The typical absorption spectrum, which was measured at a retention time of 10 min, had peaks at 211, 290 and 470 nm (Fig. 3, panel B). Absorption spectra of the brown fraction dissolved in 0.1 M sodium phosphate (pH 5.0) were similar to those shown in Fig. 3 (panel B).

Phenolic components of the brown fraction were analyzed by HPLC after acid hydrolysis. Three major components were separated (Fig. 3: Panel C, HPLC profile; panel D, absorption spectra). The component in peak 1 was identified as 2, 4, 6-trihydroxyphenylglyoxylic acid by comparing the retention time (4.5 min) and the absorption spectrum (peak; 292 nm) with synthesized 2, 4, 6-trihydroxyphenylglyoxylic acid. This compound has been reported as a component of brown onion scales (Takahama and Hirota 2000) and an oxidation product of quercetin (Barz *et al.* 1985, Schreier and Miller 1985). Retention time (7.2 min) and absorption spectrum (peaks; 204, 252 and 289 nm) of the component of peak 2 were identical to those of authentic DHBA. The presence of DHBA in dried brown onion scales has been reported by Kiviranta *et al.* (1988), Takahama and Hirota (2000) and Walker and Stahman (1955).

The component of peak 3 had a retention time of around 9 min and absorption peaks at 232, 264, 301 and 438 nm in the mobile phase [methanol-25 mM KH_2PO_4 (pH 4.6) (1 : 4, v/v)]. The retention time and absorption spectrum of this component were identical to those of P440 isolated in this study (Fig. 4, left panel). When P440 was reduced by sodium borohydride and the reduction product was analyzed by HPLC, a component, of which the retention time (Fig. 4, right) and absorption spectrum (peaks; 204, 252 and 289 nm) were identical to those of DHBA, was detected. This result suggested that P440 is *o*-quinone structure of DHBA (4-carboxy-1, 2-benzoquinone). P440 was also reduced by cysteine and mercaptoethanol but not by ascorbic acid. When the absorption spectrum of P440 was measured in 0.1 M sodium phosphate (pH 7.0), the peak was observed at about 480 nm in the visible region. This shift may have been due to dissociation of a proton from the carboxyl group.

Levels of DHBA in the brown fraction were decreased gradually to about 10% of the initial levels (175±35 nmol mg^{-1} brown fraction), whereas the levels of 2, 4, 6-trihydroxyphenylglyoxylic acid (58±4 nmol mg^{-1} brown fraction) were only slightly decreased during incubation for 100 hr in 0.1 M sodium phosphate (pH 7.0) (Fig. 5, left). During the decrease in level of DHBA, the levels of P440 initially increased and then remained nearly constant (Fig. 5, right).

Sugars

When acidic water solutions, which were obtained after ethyl acetate and butanol extraction of the acid-hydrolyzed brown fraction, were analyzed by cellulose thin-layer chromatography, a sugar was detected. This sugar had a brown color when visualized, and the R_f values were 0.28 and 0.20 in phenol saturated with water and a mixture of butanol, toluene, pyridine and water (5 : 1 : 3 : 3, v/v), respectively. The color and R_f values were identical to those of glucose. In addition to glucose, small amounts of arabinose (red in color; R_f=0.43 in phenol saturated with water and R_f=0.24 in the mixture of butanol, toluene, pyridine and water) were detected.

ESR characteristics

Not only dried brown scales (data not shown) but also the solid brown fraction isolated in this study (Fig. 6, trace A) showed a stable ESR signal and the line shape was asymmetrical. Such ESR signals were also detected when the brown fraction was dissolved in buffer solution (pH 13) under anaerobic conditions (traces B and C). The signal intensity decreased slowly during incubation in the buffer solution. Although the ESR signal of the brown fraction was also observed at pH 7.0, the intensity was quite weak. On addition of potassium hexacyanoferrate (III) to the alkaline solution of the brown fraction, new intense signals appeared. The intensity of each line decreased slowly during incubation (traces D-1 and D-2). One of the newly formed radicals (compound X) had a hyperfine structure with four lines.

To estimate the radical structure of compound X, ESR spectra of radicals of 3, 4-dihydroxybenzaldehyde, DHBA and 2, 4, 6-trihydroxyphenylglyoxylic acid were measured when they were oxidized by potassium hexacyanoferrate (III) under anaerobic conditions at pH 13. Although each main line split into two lines, the 3, 4-dihydroxybenzaldehyde radical had four main lines. The widths of the four lines were nearly identical to those among the four lines of compound X. ESR signals of DHBA radical itself were not detected. Instead, complex ESR signals were observed suggesting conversion of the benzoic acid radical into other radicals. No ESR signals were detected by the oxidation of 2, 4, 6-trihydroxyphenylglyoxylic acid.

Discussion

The result of this study showed that H_2O_2 is formed by the brown fraction isolated from dried onion scales (Fig. 1).

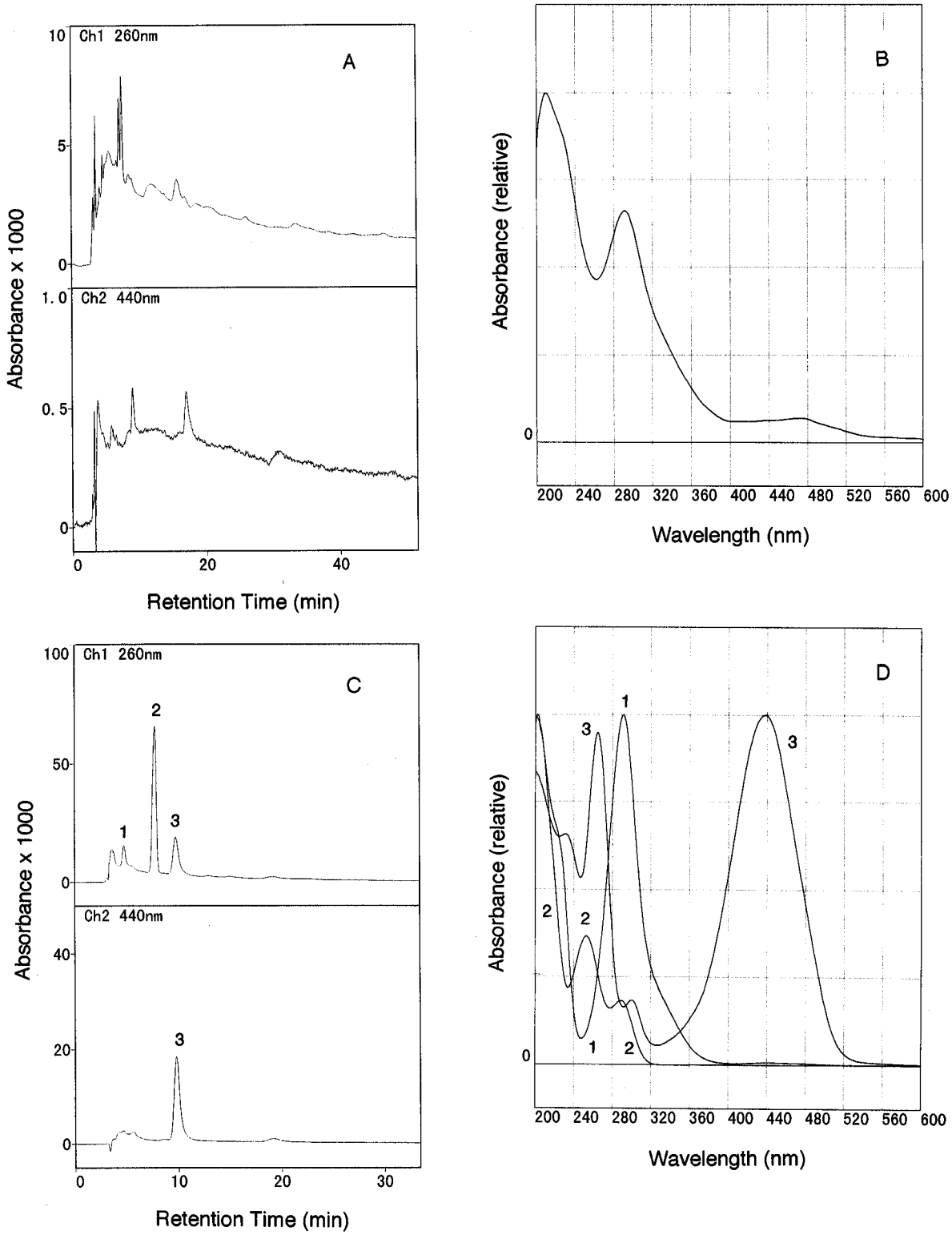

Fig. 3. HPLC of the brown fraction and the products obtained by acid hydrolysis. Panel A: HPLC profile of the brown fraction. The brown fraction (0.03 mg) was applied. Panel B: Absorption spectrum at a retention time of 10 min of the left panel in the mobile phase. Panel C: HPLC of acid-hydrolysis products (equivalent to 0.02 mg of brown fraction). Peak 1, 2, 4, 6-trihydroxyphenylglyoxylic acid; peak 2, DHBA; peak 3, P440. Panel D: Absorption spectra of peaks 1-3 of panel C in the mobile phase. Curve 1, peak 1; curve 2, peak 2; curve 3, peak 3. Conditions for HPLC: column, Shim-Pack CLC ODS; a mobile phase, methanol and 25 mM KH_2PO_4=1 : 4 (v/v); flow rate, 1 ml min^{-1}.

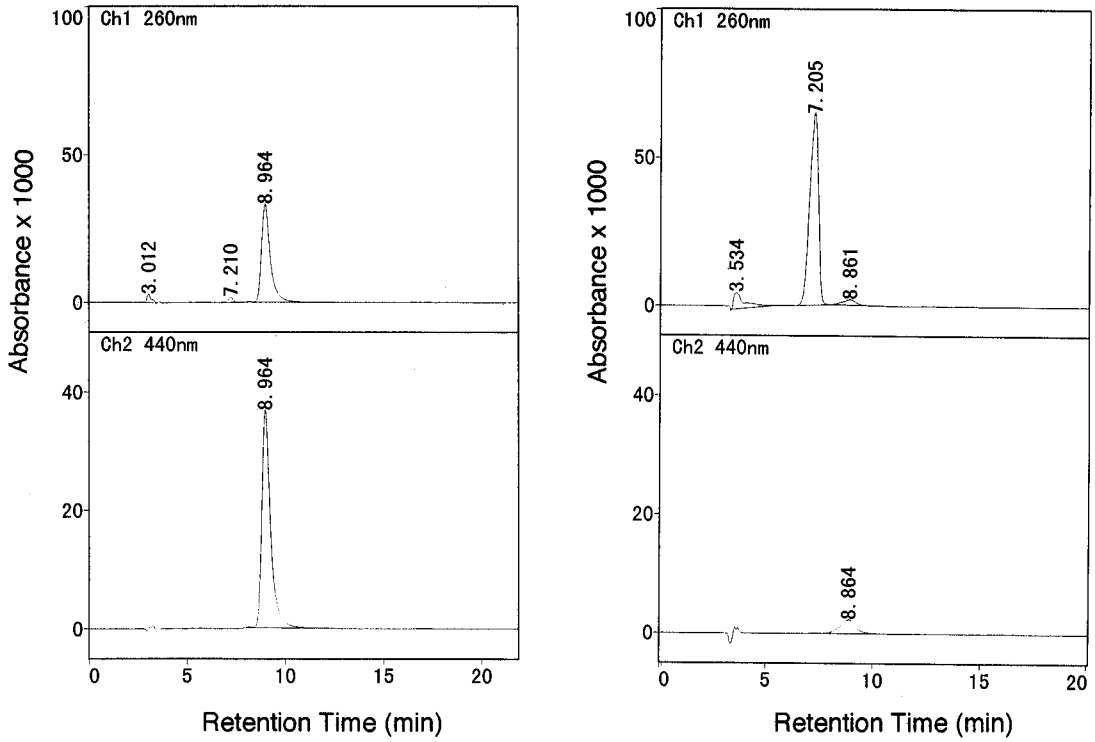

Fig. 4. P440 and the reduction product. Left: HPLC of isolated P440. Retention time and absorption spectrum of this component were identical to those of peak 3 in Fig. 3C. Right: HPLC of reduction product of P440. Conditions for HPLC: column, Shim-Pack CLC ODS; mobile phase, methanol and 25 mM KH_2PO_4=1 : 4 (v/v); flow rate, 1 ml min^{-1}. Retention time and absorption spectrum of this compound were identical to those of DHBA (peak 2 in Fig. 3C).

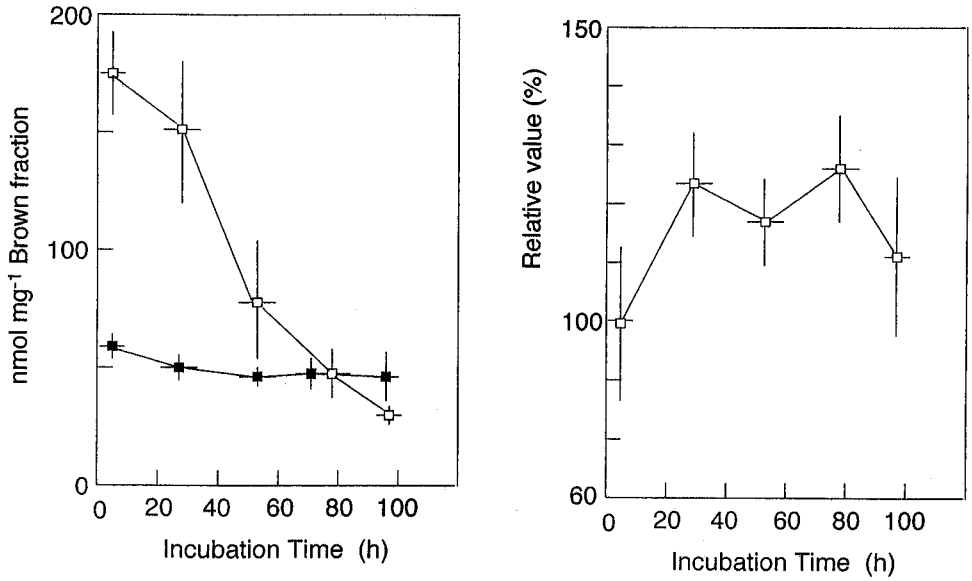

Fig. 5. Time courses of changes in levels of phenolics and the autooxidation product. Left panel. Open symbols, DHBA; closed symbols, 2, 4, 6-trihydroxyphenylglyoxylic acid. Right panel. P440.

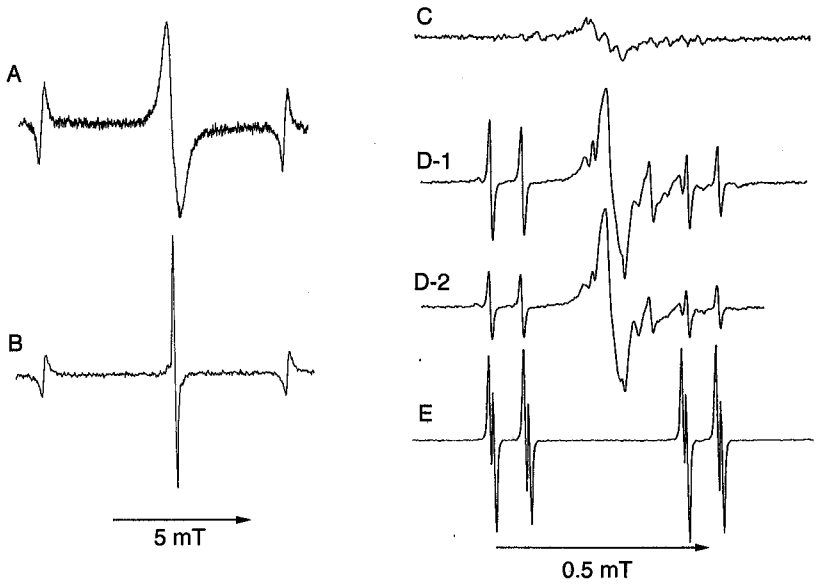

Fig. 6. ESR spectra of the brown fraction. Trace A (line width, 0.1 mT; amplification, 2,000-fold), solid brown fraction (2 mg). Trace B (line width, 0.1 mT; amplification, 1,000-fold), 9.2 mg ml^{-1} of the brown fraction in 0.2 M KCl-0.2 M NaOH buffer (pH 13). Two peaks of both sides of traces A and B are signals of MnO_2. Trace C (line width, 0.01 mT; amplification, 1,000-fold), 9.2 mg ml^{-1} of the brown fraction in 0.2 M KCl-0.2 M NaOH buffer (pH 13). Traces D-1 and D-2 (line width, 0.01 mT; amplification, 500-fold), 12 mg ml^{-1} of the brown fraction plus 6 mg ml^{-1} of potassium hexacyanoferrate (III) in 0.2 M KCl-0.2 M NaOH buffer (pH 13). Trace E (line width, 0.005 mT; amplification, 200-fold), 9.8 mg ml^{-1} of 3, 4-dihydroxybenzaldehyde plus 5.4 mg^{-1} of potassium hexacyanoferrate (III) in 0.2 M KCl-0.2 M NaOH buffer (pH 13). Traces D-1 and D-2 were recorded 5 and 10 min after preparation of the reaction mixture.

About 50% of consumed molecular oxygen was transformed to H_2O_2. During H_2O_2 formation, absorption at 495 nm increased (Fig. 2). These results suggested that the brown fraction contains autooxidizable components. As DHBA and 2, 4, 6-trihydroxyphenylglyoxylic acid were contained in the brown fraction (Fig. 3), these phenolic acids are candidates for autooxidation. The time courses of decreases in levels of the phenolics were measured during autooxidation, and level of DHBA but not 2, 4, 6-trihydroxyphenylglyoxylic acid was shown to decrease significantly (Fig. 5). This result suggested that DHBA is preferentially oxidized by molecular oxygen between the two phenolics. The mechanisms responsible for the oxidation remain to be elucidated.

As the level of P440 increased initially during autooxidation of the brown fraction (Fig. 5) and P440 had a peak at about 480 nm at pH 7.0, the absorption increase at 495 nm observed during autooxidation of the brown fraction (Fig. 2) may be, in part, due to the formation of P440 by oxidation of DHBA that binds to the brown fraction. The time course of the decrease in the level of DHBA was different from those of increases in P440 (Fig. 5). From this result, we deduced that after DHBA has been transformed to P440, the product is converted to other as yet unidentified components.

When sodium borohydride was added to the brown fraction, absorbance around 490 nm decreased (Fig. 2). The decrease in absorbance was, in part, due to reduction of P440 of the brown fraction, as P440 had a peak at about 480 nm in 0.1 M sodium phosphate (pH 7.0). The decrease in absorbance at 295-330 nm (Fig. 2) may be, in part, due to reduction of 2, 4, 6-trihydroxyphenylglyoxylic acid of the brown fraction. This was deduced from the observation that this compound had absorption peaks at about 295 and 320 nm at pH 7.0 and that the degree of absorption of the peaks were decreased by sodium borohydride. As a keto group can be reduced by sodium borohydride, the keto group of the 2, 4, 6-trihydroxyphenylglyoxylic acid moiety of the brown fraction may be reduced by the reductant.

A mixture of *p*-hydroquinone and *p*-benzoquinone forms a charge-transfer complex (quinhydrone structure) in aqueous solution, and the structure is a stable radical. The brown fraction had stable radicals and the line shapes were asymmetrical (Fig. 6, traces A-C). It has been reported that quinhydrone type structures have such ESR characteristics (Chio *et al.* 1982, Oniki and Takahama 1992). The observation that radical intensities of the brown fraction were much stronger at pH 13 than at pH 7 supported the presence of quinhydrone type structures in the brown fraction, because quinhydrone type structures are preferentially formed under alkaline conditions (Arnaud *et al.* 1983, Chio *et al.* 1982). P440 and DHBA and/or 2, 4, 6-trihydroxyphenylglyoxylic acid may behave as quinone and hydroquinone forms, respectively, in the formation of quinhydrone type structures in the brown fraction. Glucose and arabinose were also found in the acid-hydrolyzed brown fraction. Further studies are required to elucidate the chemical structure of the brown fraction and the role of the quinhydrone type structure in oxygen uptake.

Dried brown outer scales of yellow onion bulbs as well as

the brown fraction produced H_2O_2 when suspended in a buffer solution. As H_2O_2 is normally formed from O_2^-, the formation of H_2O_2 suggested that in mature onion bulbs, reactive oxygen species are formed in the outer brown scales in the growing soil. DHBA in brown scales is antimicrobial (Friend 1979, Walker and Stahman 1955) and the quinone body of DHBA may also be antimicrobial as quinones (phaselic acid and *trans*-clovamide) participate in resistance of red clover to infection by *Kabatiella caulivora* (Friend 1979) and quinones formed from *o*-dihydroxyphenols inhibit enzymes with functional SH groups (Edreva 1999). From the results of the present study, we concluded that yellow onion bulbs are protected from infection in the growing soil by forming antimicrobial phenolics and reactive oxygen species.

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