Betalains—A New Class of Dietary Cationized Antioxidants

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Antioxidant nutrients from fruits and vegetables are believed to be a class of compounds that exert their effects in humans by preventing oxidative processes which contribute to the onset of several degenerative diseases. This study found a new class of dietary cationized antioxidants in red beets (*Beta vulgaris* L.). These antioxidants are betalains, and the major one, betanin, is a betanidin 5-*O*-*β*-glucoside. Linoleate peroxidation by cytochrome *c* was inhibited by betanin, betanidin, catechin, and α-tocopherol with IC_{50} values of 0.4, 0.8, 1.2, and 5 μM, respectively. In addition, a relatively low concentration of betanin was found to inhibit lipid peroxidation of membranes or linoleate emulsion catalyzed by the "free iron" redox cycle, H_2O_2-activated metmyoglobin, or lipoxygenase. The IC_{50} inhibition of H_2O_2-activated metmyoglobin catalysis of low-density lipoprotein oxidation by betanin was <2.5 μM and better than that of catechin. Betanin and betanidin at very small concentrations were found to inhibit lipid peroxidation and heme decomposition. During this reaction, betanidin was bleached completely, but betanin remained unchanged in its absorption. This difference seems to derive from differing mechanisms of protection by these two compounds. The high affinity of betanin and betanidin for membranes was demonstrated by determining the rate of migration of the compounds through a dialysis tube. Betanin bioavailability in humans was demonstrated with four volunteers who consumed 300 mL of red beet juice, containing 120 mg of the antioxidant. The betacyanins were absorbed from the gut and identified in urine after 2−4 h. The calculated amount of betacyanins found in the urine was 0.5−0.9% of that ingested. Red beet products used regularly in the diet may provide protection against certain oxidative stress-related disorders in humans.

Keywords: *Antioxidants; betalains; cationized; red beet; bioavailability; human*

INTRODUCTION

Epidemiological studies strongly suggest that high intakes of vegetables and fruits reduce the risk of some diseases such as atherosclerosis and cancer (*1*−*5*). It has been postulated that the generation of free radicals, among them reactive oxygen species, is associated with cellular and metabolic injury. These observations have sparked a great interest in the use of antioxidants that could prevent in vivo oxidative damage. Currently, there is considerable interest in elucidating the role of dietary antioxidants in human nutrition and medicine. Most of the research has been focused on dietary antioxidants such as ascorbic acid, α-tocopherol, and carotenoids (*7*−*10*). Recently, flavonoids and phenolic compounds have gained much interest as dietary antioxidants (*11*−*17*). These compounds often exhibit antioxidant activity, and the potential health benefits of fruits, vegetables, green tea, and red wine might be partly due to this property of the flavonoids and other phytochemicals. It is noteworthy that, to date, we have not identified all of the compounds which may function as dietary antioxidants.

The bioavailability of the flavonoid class is an important unsolved problem in evaluating its many alleged health effects. Several authors have determined the antioxidant properties of many plants (medicinal plants, fruits, and vegetables) without, however, taking into consideration their bioavailability in humans (*18*−*26*). Several studies consider the bioavailability of several classes of flavonoids in human, but the amounts found in the plasma were relatively low (*27*−*37*).

Betalains include two classes of compounds: betacyanins, which are red violet, and betaxanthins, which are yellow. Of the numerous natural sources of betalains, red beet and prickly pear are the only foods containing this class of compounds (*38*, *39*). The major betalain in red beets is betanin, which is a betanidin 5-*O*-*β*-glucoside (Figure 1) containing a phenolic and a cyclic amine group, both shown by us to be very good electron donors, acting as antioxidants (*40*).

Several studies have presented data showing that red beet is a good source of natural antioxidants (*21*, *25*, *26*, *40*−*42*). The objectives of the present study were to evaluate the potential antioxidant activity of betalains from red beet and to determine their potential bioavailability in humans.

MATERIALS AND METHODS

Materials. Metmyoglobin (type 1) from equine skeletal muscle, soybean lipoxygenase type I, cytochrome *c* (from horse heart; cyt *c*), ascorbic acid, α-tocopherol, catechin, and thiobarbituric acid were obtained from Sigma Chemical Co. (St. Louis, MO) and hydrogen peroxide (30%) and trichloroacetic acid from Merck (Darmstadt, Germany). The dialysis tube, Visking size 3−20/32 in., was from Medicell International Ltd. (London, U.K.).

Fresh red beets were purchased from an outdoor market.

Methods. *Fractionation of Betalains by Liquid Chromatography (LC) on Sephadex G-25.* Red beets were heated by

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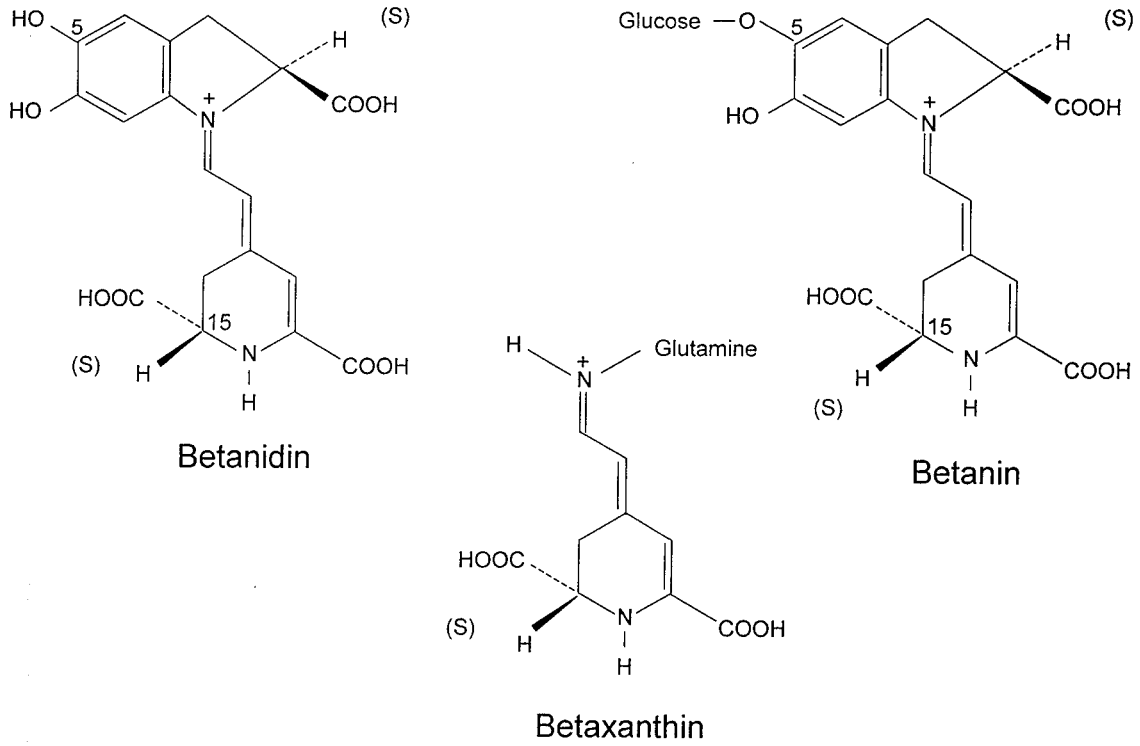

Figure 1. Chemical structures of betanin and betanidin.

microwave for 5 min at 98 °C, cooled to 25 °C, and then crushed in a Waring blender for 1 min. The pulp was centrifuged at 10000*g* for 15 min at 4 °C, and the supernatant was collected for betalains estimation and for separation on a Sephadex G-25 column (30 cm × 2 cm). A 1-mL sample of the supernatant was eluted with acetic acid (1%), and 3-mL fractions were collected and tested for betaxanthin at 478 nm, betanins at 536 nm, and betanidins at 542 nm. Betalain from red beets was separated by gel filtration into three main fractions: (a) betaxanthin, (b) betanin, and (c) betanidin. Pure betanin and betanidin were prepared from the fractions collected after gel filtration and crystallization according to a method described by Schwartz et al. (*38*). The material collected was used for the identity and quantity calibration of high-performance liquid chromatography (HPLC) using extinction coefficient values as described below. The same material was used for the evaluation of the antioxidant activity. Betanidin was prepared from juice, which was treated at 25 °C for 3 h by 5% cellulase (Celluclast L5-Novo Novodisk Ferment) having β-glucosidase activity. The concentrations were estimated using ϵ values of 65000 for 1 M betanin, 54000 for betanidin, and 60000 for betaxanthin (*38*).

HPLC Identification of Betalains from Red Beet Juice and Urine. Red beet juice, LC fractions, and urine were run by HPLC at ambient temperature. The HPLC separation was done on a column of Waters Novapak C-18, 3.9 × 150 nm, connected to a Merck Hitachi pump, L6200A, and a Jasco Uvidec-100-IV UV−vis detector. The eluents were solvent A, CH_3OH/0.5 M KH_2PO_4, (18:82), and solvent B, CH_3OH, with a gradient transition from 100% solvent A to 50% solvent B during 15 min. The chromatograms were monitored at 536 nm. The betanin and betanidin fractions were further separated by HPLC as described by Schwartz et al. (*38*).

Lipid Peroxidation and Antioxidation Activities. (a) Linoleate Peroxidation. The assay of linoleate diene conjugation was carried out as previously described (*14*). The technique consists of following the increase in conjugated dienes by their absorption at 233 nm. The test sample contained 1.5 mL of buffered linoleate at pH 7.0, active fraction 0.1−0.4 mL, and distilled water in a mixture as follows: linoleate, 2 mM; linoleate hydroperoxide, 2−4 μM; Tween 20, 0.05%; phosphate buffer, 0.05 M, pH 7.0; diethylenetriaminepenta-acetic acid (DETA), 0.5 mM. The blank sample contained all of the reagents except the catalyzers. Linoleate hydroperoxides were calculated using a molar extinction coefficient of $E_{234} = 25.500$ (*43*). Lipid peroxidation of this system was catalyzed by cytochrome *c*, metmyoglobin, and lipoxygenase, with or without the presence of several antioxidants.

(b) Low-Density Lipoprotein (LDLs) Oxidation. LDL was prepared from blood collected in EDTA by venipuncture (1.5 mg/mL) from four nonsmoking healthy male volunteers, as described previously (*11*). Prior to the oxidation experiments, LDLs were extensively dialyzed with deoxygenated phosphate-buffered (10 mM, pH 7.4) saline (100 mM) for 24 h. The final concentration of each sample was adjusted to the same protein content (0.25 mg/mL LDL) by addition of phosphate-buffered saline (10 mM). The oxidative susceptibility of LDL was evaluated by determining oxygen absorption during lipid peroxidation in a reaction system containing activated metmyoglobin/H_2O_2 (30 μM each) using an oxygen monitor (Yellow Spring Instrument Co. model 53) with a Clark electrode.

(c) Microsomal Lipid Peroxidation. Isolation of the microsomal fraction from turkey muscle tissue was done as described previously (*43*). Microsomes for lipid peroxidation assays were incubated in air in a shaking water bath at 37 °C. The reaction mixture contained microsomes at 1 mg of proteins/mL and 4 mL of 50 mM acetate buffer, pH 7.0. Protein determination assays were conducted according to the modified Lowry procedure (*44*) with bovine serum albumin as standard.

The thiobarbituric acid reactive substances (TBARS) were determined according to the method of Bidlack et al. (*45*). The results presented are the means of triplicates, and in the figure each bar (I) denotes the standard deviation.

Dialysis Experiments. Betanin and betanidin with or without the addition of microsomes were inserted into a dialysis tube containing 5 mL of 0.05 M phosphate buffer at pH 7.0. Before dialysis, the concentration of betanin and betanidin was 30 μM, and the microsomal concentration was 2 mg of protein/mL. The compounds were dialyzed against 10 mL of the same buffer; betanin and betanidin outside the dialysis tube were determined spectrophotometrically at 536 nm.

Production of Red Beet Juice Enriched with Betanin and Betanidin following Enzymatic Treatment. Red beet juice was produced from raw material heated at 98 °C for 30 min and peeled with a carborundum peeler. The red beets were cooled to room temperature by immersion in tap water, and the juice

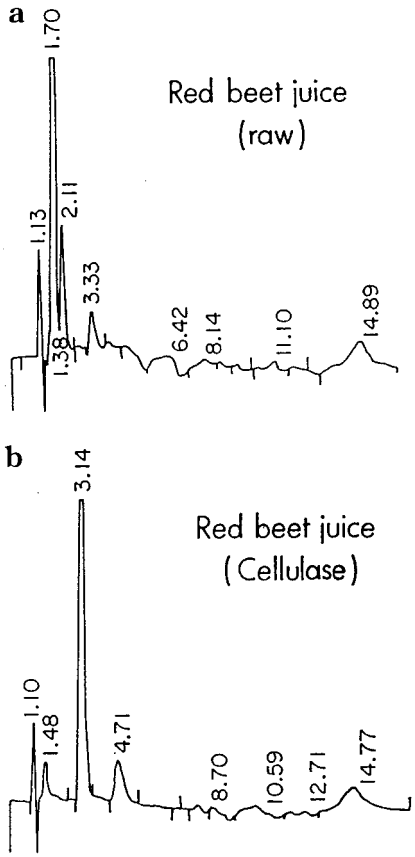

Figure 2. HPLC chromatogram of red beet juice: (a) raw juice; (b) cellulase treated (5%, at 25 °C for 30 min). Detection was at 536 nm.

was extracted with a "Santos" juice extractor and mixed for 30 min at room temperature with the pulp and pectolytic and cellulolytic enzymes (Novo, Novodisk Ferment), Pectinex Ultra JP-L and Celluclast L5, each at a concentration of 0.1%.

Following the enzymatic treatment, the juice was filtered through a Brawn separator and pasteurized with a plate pasteurizer at 98 °C for 10 s, for hot filling into 250-mL glass bottles. The bottles were cooled to room temperature under tap water. This treatment reduced the pulp content from 35 to 7% and increased the juice extraction from 64 to 92% (*40*).

Nutritional Study Design. Four healthy, fasting individuals, aged 35−55, participated as subjects (two females and two males) in the study, which was carried out at our laboratory. On each of the experimental days, they did not consume any kind of product rich in polyphenols (vegetables, fruits, tea, red wine, etc.). On the control day, every hour for 12 h, they drank 200 mL of water and collected urine into sterile tubes, which were frozen immediately (−20 °C). Three weeks later, on the first test day, they repeated the same procedure but replaced the first dose of water with 300 mL of red beet juice, containing 120 mg of betanin. The urine was collected into sterile tubes during 12 h and frozen at −20 °C. Each urine tube was determined with a creatinine test kit (Sigma Diagnostics) for evaluation of the concentration of the betacyanins on the same basis.

RESULTS

Red beets contain a large concentration of betanin, 300−600 mg/kg, and lower concentrations of isobetanin, betanidin, and betaxanthins.

The red beet juice chromatogram after HPLC separation (Figure 2a) shows the presence of betanin, isobetanin, and betanidin with retention times of 1.70 ± 0.06, 2.11 ± 0.06, and 3.33 ± 0.08 min, respectively. Incubation of the juice with the enzyme cellulase, which

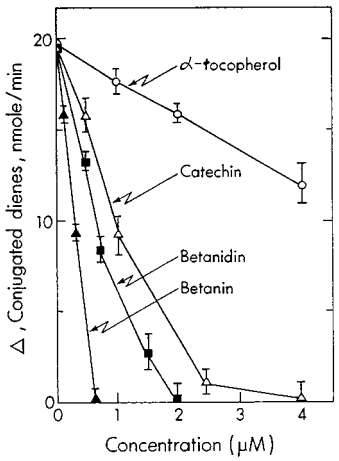

Figure 3. Inhibition of linoleate peroxidation by betanin, betanidin, catechin, and α-tocopherol induced by cytochrome *c* (0.25 μM) at pH 7.0 and 25 °C.

contains β-glucosidase activity, produced betanidin and isobetanidin with retention times of 3.14 ± 0.05 and 4.71 ± 0.06 min, respectively (Figure 2b).

Linoleate peroxidation by cyt *c* was inhibited by very low concentrations of betanin (Figure 3). The IC_{50} inhibition concentrations of linoleate peroxidation by betanin, betanidin, catechin, and α-tocopherol were 0.4, 0.8, 1.2, and 5 μM, respectively. Betaxanthins were also found to inhibit lipid peroxidation by cyt *c* at an IC_{50} of ~1.0 μM (results not shown).

Lipid peroxidation of linoleic acid was also catalyzed by metmyoglobin (Figure 4A). During this reaction, linoleate oxidation products, which absorb at 234−240 nm and at 282 nm, increased and the Soret spectrum at 408 nm decreased, indicating oxidation and breakdown of the heme structure. The addition of betanin to this system (Figure 4B) prevented oxidation of the heme structure and accumulation of conjugated dienes and oxidation products at 282 nm. Pigment absorption was registered at 536 nm and was found to be stable during the oxidation process. The addition of betanidin to the same system prevented the oxidation of the heme structure and accumulation of conjugated diene and oxidation products at 282 nm, but during this process the spectrum at 542 nm decreased, indicating destruction of the pigment conjugated double-bond structure (Figure 4C).

LDL oxidation by H_2O_2-activated metmyoglobin was determined by oxygen absorption with a Clark electrode. The IC_{50} inhibition concentration for LDL oxidation by betanin was <2.5 μM and better than that of catechin, a well-known antioxidant (Figure 5). The study on microsomal lipid peroxidation demonstrated (Table 1) that the inhibitory effect of betanin depended very much on the catalyst used in the system. In the presence of ascorbic acid−$FeCl_3$ (AA−Fe) a low concentration of betanin acted pro-oxidatively and only 25 μM of betanin inhibited lipid oxidation significantly. However, when H_2O_2-activated metmyoglobin was the catalyzer, <5 μM almost totally inhibited membrane oxidation.

Lipoxygenase (soybean type I; 0.03 unit) oxidized linoleate at a rate of 30 μmol/min, producing ~10 μM linoleate hydroperoxides. The IC_{50} values of lipoxygenase by betanidin, betanins, and catechin were found to be 0.3, 0.6, and 1.2 μM, respectively (Figure 6).

The affinity of betanidin to the membrane of microsomes is demonstrated in Figure 7. The diffusion of

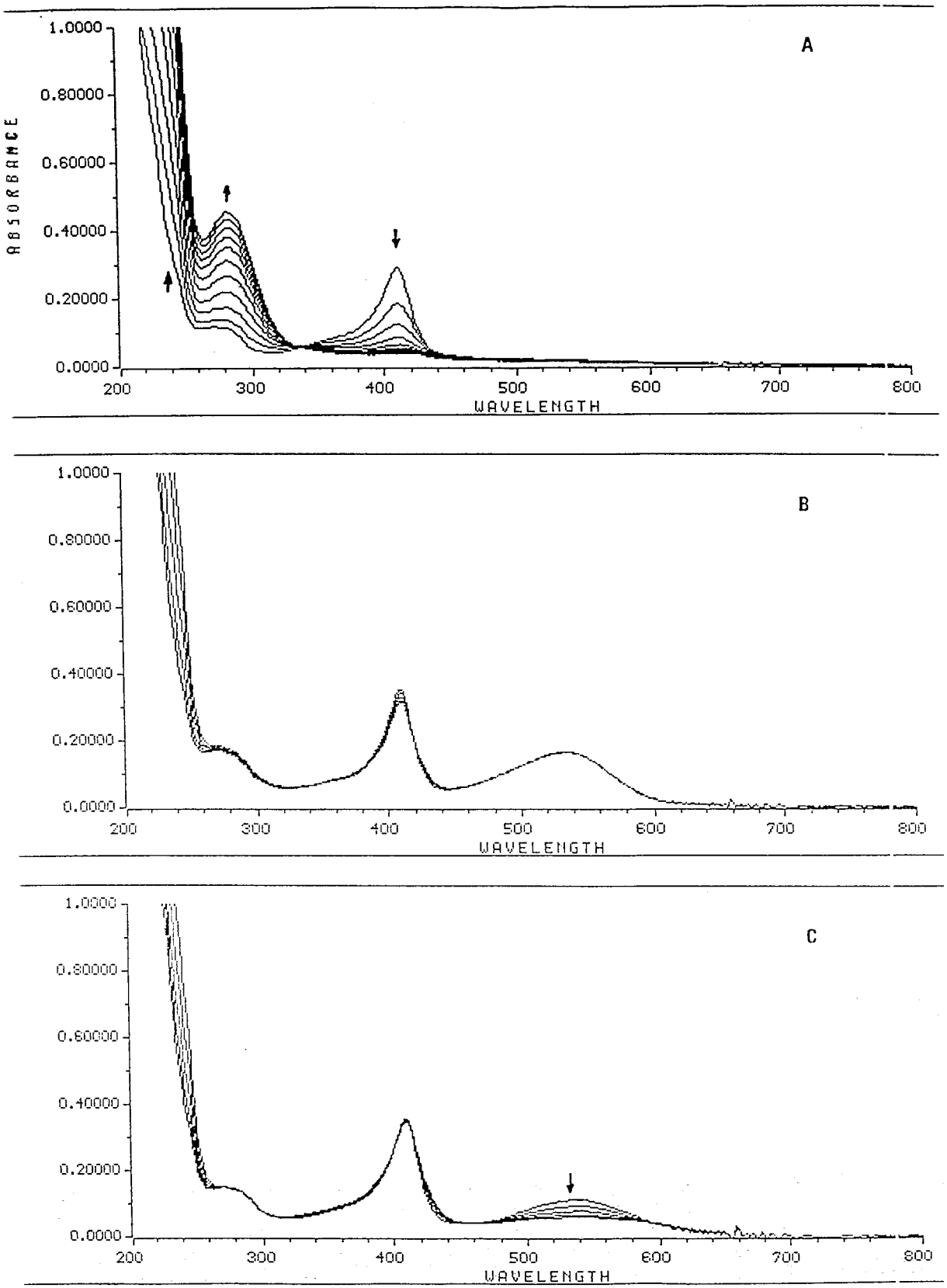

Figure 4. Spectral changes during linoleate peroxidation by metmyoglobin (2.5 μM) inhibited by betanin and betanidin at 1.92 μM, pH 7.0, and 25 °C: (A) control; (B) betanin; (C) betanidin. Each line denotes a 30 s interval.

betanin and betanidin from the dialysis tube in the presence of microsomes was significantly lower than that from the same system without microsomes.

The bioavailability of betanin was determined with four volunteers, who consumed a polyphenol-free diet and drank 300 mL of red beet juice containing 120 mg of betanin. The volunteers drank 200 mL of water every hour for 12 h, and their excretion of betanin or its isomer was determined spectrophotometrically and after HPLC separation. A typical chromatogram of urine from a

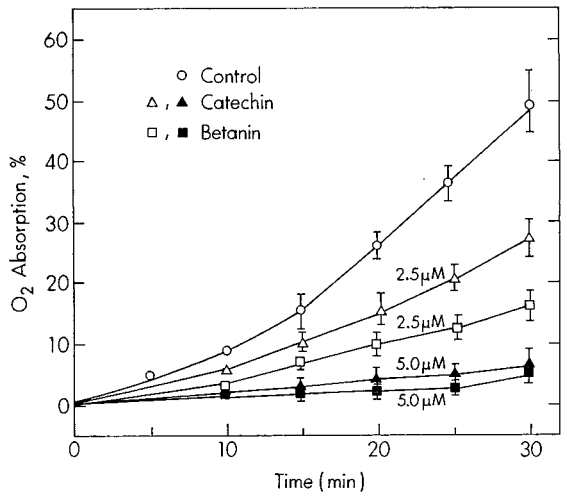

Figure 5. Inhibition of LDL (0.25 mg/mL) oxidation by catechin and betanin, induced by H_2O_2-activated metmyoglobin (30 μM each) at pH 7.4 and 25 °C.

Table 1. Inhibition by Betanin of Micrsomal Lipid Peroxidation Catalyzed by Different Catalyzers[a]

betanin concn (μM)	treatment	
	AA−Fe	Mb−H_2O_2
	TBARS 532 nm	
0	0.205 ± 0.02	0.230 ± 0.04
5.0	0.310 ± 0.03	0.015 ± 0.02
12.5	0.320 ± 0.01	0.012 ± 0.01
25.0	0.019 ± 0.01	0.014 ± 0.01

[a] Catalyst concentrations: AA−Fe (200 μM ascorbic acid, 5 μM $FeCl_3$); Mb−H_2O_2 (30 μM Mb, 10 μM H_2O_2), and microsomes 1 mg of protein/mL, at pH 7.0 after incubation at 25 °C for 60 min.

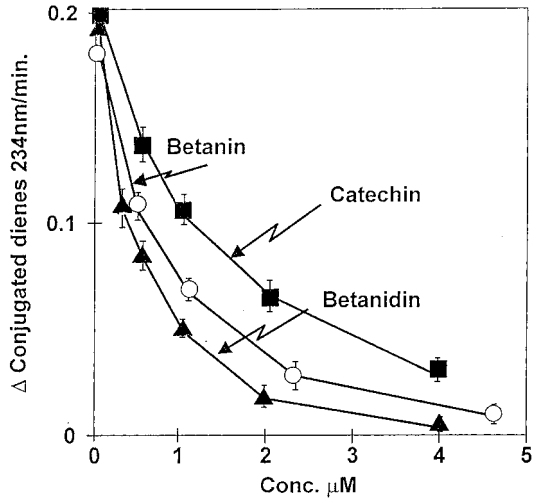

Figure 6. Inhibition of lipoxygenase (0.03 unit) as affected by betanin, betanidin, and catechin concentration, at pH 7.0 and 25 °C.

volunteer, after ingestion of red beet juice, is presented in Figure 8. The chromatogram shows two peaks at retention times of 2.08 and 3.01 min. The first peak is identical to that of isobetanin. The betacyanins were absorbed from the gut into the blood system, and it was possible to identify them in urine after 2−4 h. The excretion kinetics during the 12 h of the experiment varied among the volunteers. Two representative diagrams are presented in Figure 9. Each of the volunteers ingested 120 mg of betanin, and the calculated amount of betacyanins in the urine, based on an extinction

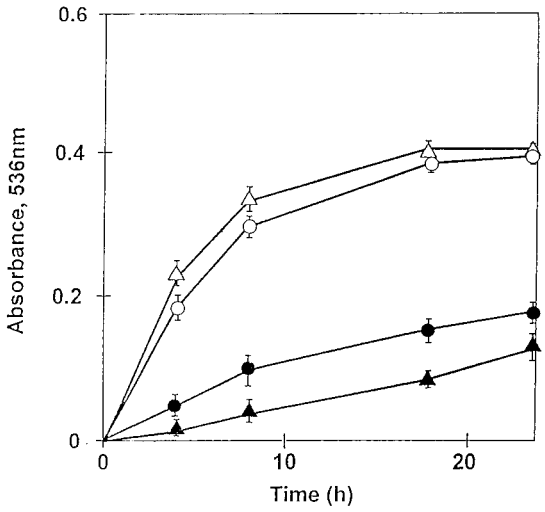

Figure 7. Diffusion of betanin and betanidin (30 μM) through a dialyzing tube with or without microsomes at a concentration of 2.0 mg of protein/mL in buffer phosphate, 0.05 M, pH 7.0, at 4 °CL (○) betanin; (△) betanidin; (●) betanin with microsomes; (▲) betanidin with microsomes.

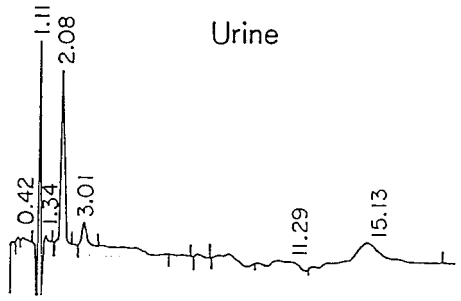

Figure 8. HPLC chromatogram of human urine after consumption of red beet juice.

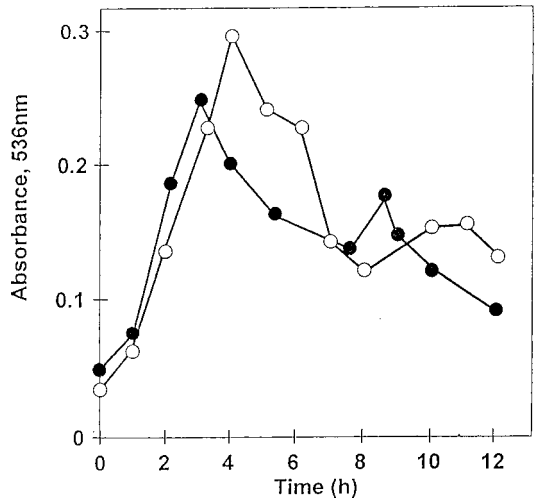

Figure 9. Betacyanins excreted in urine of volunteers after consumption of 300 mL of red beet juice: (○) volunteer 1; (●) volunteer 2.

coefficient of 65000 for betanin and isobetanin, was 0.60−1.08 mg, or 0.5−0.9% of that ingested.

DISCUSSION

There is increasing evidence that oxidative stress leads to many biochemical changes, which are important contributor factors in the development of several human chronic diseases. Antioxidant enzymes, complemented

by small molecule antioxidants, some of which are derived from the diet, protect against the potentially injurious effects of oxygen radicals.

Our previous study (*40*) and the present one demonstrated for the first the strong antioxidant effect of betacyanins and their bioavailability in humans. Betacyanins are not flavonoids, but they contain a partly glucosidized phenolic group. Betalains, in addition, contain a cyclic amine group, the structure of which resembles that of ethoxyquin, a very strong antioxidant used to stabilize lipids in animal feeds (*46*, *47*).

The iron in cyt *c* is bound at all six coordination sites. Iron cyt *c* interacts with the lipid hydroperoxide without producing an intermediate compound such as ferryl (*48*). We assume that ferric cyt *c* catalyzed lipid peroxidation by reactions 1 and 2 (*14*).

$$\text{cyt } c\ Fe^{3+} + ROOH \rightarrow \text{cyt } c\ Fe^{2+} + ROO^{\bullet} + H^{+} \quad (1)$$

$$\text{cyt } c\ Fe^{2+} + ROOH \rightarrow \text{cyt } c\ Fe^{3+} + RO^{\bullet} + HO^{-} \quad (2)$$

In these reactions betacyanin may prevent lipid peroxidation by interacting with peroxyl and alkoxyl radicals. Betanin was found to act better than betanidin, catechin, or α-tocopheorl, most probably because of its high solubility in the aqueous system.

Betacyanins were also found to act as very efficient antioxidants in a model system containing linoleate peroxidized by metmyoglobin. In this model system we determined four parameters of oxidation: (a) accumulation of conjugated dienes at 240 nm; (b) accumulation of oxidation products at 282 nm [these oxidation products were identified by us previously (results not shown) as ketodienes, by using a method of Wu and Robinson (*49*)]; (c) decomposition of heme at 408 nm; and (d) breakdown of the pigment structure.

Betanin at a small concentration was found to inhibit lipid peroxidation and heme decomposition. At the same concentration the aglycon of betanin, betanidin, exhibited almost the same effect in preventing lipid peroxidation. During this reaction, betanidin was bleached completely, at 542 nm, but betanin remained unchanged in its absorption at 536 nm.

These differences seem to derive from differing mechanisms of protection by these two compounds. Betanin, which contains a glucose at the ortho position to the hydroxyl, seems to prevent the phenolic group from interacting with the lipid radical, giving the amine group the chance to work as a hydrogen donor, to form a relatively stable radical or a dimer without changing the absorption peaks. This assumption is based on the reaction of ethoxyquin and its active cyclic amine during oxidation (*50*, *51*) and the antioxidative activity of betaxanthins, which contains only a cyclic active amine. However, all of these reactions for betanin need to be proven in the future.

Betanidin, which is an *o*-diphenol, acts as an antioxidant and during this reaction seems to form a phenoxyl radical, semiquinones, and polymeric compounds, which lost color at 542 nm. Betanin, which, like catechin, is soluble in water, was almost twice as effective as catechin in preventing LDL oxidation by H_2O_2-activated metmyoglobin. This effect seems to derive from the high affinity of betanin to membranes shown in Figure 7. Betacyanins are cationized compounds with a high affinity to the negative charges of membranes, and this affinity prevents their rate diffusion through the dialyzing tube. It has been shown that cationization of enzymes such as catalase, which increases the affinity to membranes, is beneficial in preventing oxidative stress in rat intenstinal epithelium or damage to *Escherichia coli* cells (*52*, *53*). In a model system of membrane lipid peroxidation we compared betanin activities promoted by two catalysts: "free iron" (AA−Fe) and H_2O_2-activated metmyoglobin. In the system catalyzed by H_2O_2-activated metmyoglobin, betanin was effective at concentrations $<5\ \mu$M, whereas in the system catalyzed by AA−Fe, an iron redox cycle, the effectiveness was exhibited only at a concentrations $>25\ \mu$M. At lower concentrations betanin was pro-oxidative.

This dramatic change in betanin antioxidant activity could be accounted for by its electron-donating capability to enhance the reduction of ferric to ferrous ions, so producing more pro-oxidative reactive compounds and enhancing breakdown of hydroperoxides. This process could be made to decline only by increasing the concentration of betanin 5-fold. At high concentrations the antioxidant works also with lipid free radicals, thus preventing lipid peroxidation. A similar effect was achieved by us previously with catechin or quercetin (*30*).

Betanidin and betanin were found to inhibit soybean lipoxygenase at very low concentrations, with IC_{50} values of 0.25 and 0.5 μM, respectively. Catechin was found to work as an inhibitor of soybean lipoxygenase with an IC_{50} of 1.1 μM. It seems that the strong inhibition of soybean lipoxygenase by betanin and betanidin was achieved through reduction of iron to the ferrous inactive form. It has already been proposed that the capacity to reduce the iron ion determines the ability of some phenolic compounds to inhibit soybean lipoxygenase type I (*54*, *55*) or 5-lipoxygenase (*56*).

It is also possible that betanidin and betanin interact with the enzyme peroxyl radical complex through a reaction which prevents the activation of the enzyme:

$$\text{Enz-}Fe^{2+} - LOO^{\bullet} + BH \rightarrow \text{Enz-}Fe^{2+} + LOOH + B^{\bullet} \quad (3)$$

One of the most important questions concerning the in vivo activity of many antioxidants found in fruits and vegetable is their bioavailability from the diet, through the gut into the blood system. Our present study yielded data demonstrating the partial absorption of betacyanins in humans: a significant increase in the urine absorption at 536 nm was detected 2−3 h after ingestion of 300 mL of red beet juice.

In urine, the major peak was identified as isobetanin. It may be that betanin was isomerized to isobetanin at the relatively high temperature of the body, in accordance with Jackman and Smith's (*39*) finding that high temperatures could isomerize betanin. The total amount of isobetanin collected in urine was ∼0.6−1.0 mg, which represents 0.5−0.9% of the ingested betacyanins.

The results regarding betanin absorption support those of Hollman's group (*27*) on the bioavailability of quercetin 5-glucosides from onion and their speculation that intestinal glucose carriers may play a role in this absorption. Most recently, this hypothesis was supported by Gee et al. (*57*), who found that quercetin glucosides are capable of interacting with the sodium-dependent glucose transport receptors in the mucosal epithelilum and may therefore be absorbed by the small intenstine in vivo.

Recent studies have demonstrated that dietary flavonoids can be absorbed and enter the blood circulation (*27*, *28*, *30*−*32*), although there is controversy about the intestinal absorption of the intact form of flavonoids (*17*).

Several researchers have shown that a great part of the flavonoids absorbed in the blood are present as conjugated derivatives, mostly as glucoronides and sulfate conjugated (*31*, *58*, *60*). We have no evidence about conjugation of betacyanins. Nevertheless, the absorption of betacyanins in humans was found to be low, although very similar to that of flavonoids as found in several studies (*29*−*32*, *34*, *35*, *60*).

Recent studies have demonstrated that the concentration of flavonoids in the plasma could reach ∼1 μM or below. The general antioxidant level in human plasma can reach a concentration of ∼800−1000 μM, much higher than those found for flavonoids or betacyanins. For this reason, it seems that if natural antioxidants such as betalains or flavonoids partially prevent the process of atherosclerosis or other diseases, they should act at low concentration and very specifically.

More important, ∼99% of those compounds remain in the gastrointestinal area, and they may alleviate several diseases that are affected by oxidative stress (*61*, *62*). As the betalains are cationized compounds, their affinity for membranes may improve their activity similar to that found for cationized catalase (*52*).

In conclusion, our study demonstrated the strong antioxidant effects of betacyanins in model systems of lipid peroxidation catalyzed by cytochrome *c*, H_2O_2-activated metmyoglobin, iron redox cycle, and lipoxygenase, in microemulsions, membranes, and LDL. This class of compounds is cationized, which increases their affinity to membranes, a great beneficial attribute for antioxidants. The study also demonstrated that betacyanins are absorbed from the gut and that they reach the urine in amounts of ∼1% of those ingested, similar to several flavonoids. Red beet juice and other red beet products used regularly in the diet may provide protection against certain oxidative stress-related disorders in humans.

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