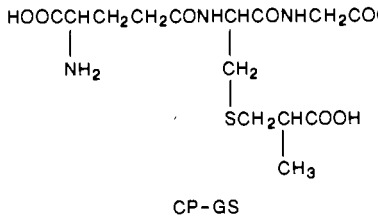
S-(2-Carboxypropyl)glutathione in Vegetables of Liliflorae

Seiji Tsuboi, Satomi Kishimoto, and Shinji Ohmori*

A high-performance liquid chromatographic determination of S-(2-carboxypropyl)glutathione (CP-GS) in vegetables was established. The procedure is based on the conversion to a 2,4-dinitrophenyl derivative and detection at 380 nm. By this method CP-GS could be determined in the range of 44 pmol to at least 4.4 nmol. The recovery of CP-GS added to onion homogenate was 91.3 ± 3.8%. By this method, CP-GS was determined in various vegetables (onion, garlic, narcissus, yellow leek, green leek, wakegi, spring onion), and some biochemical studies of it were carried out.

In 1960 CP-GS (S-(2-carboxypropyl)glutathione) was isolated from onion (Virtanen and Matikkala, 1960) and also from garlic (Suzuki et al., 1961a). S-(2-Carboxypropyl)cysteine (CP-Cys), a sulfur-containing amino acid, was found in human urine by Mizuhara and Ohmori (1961) and in onion by Granroth and Virtanen (1967). Suzuki et al. (1962a) described active incorporation of [^{14}C]valine into CP-GS in excised root of garlic and assumed the carboxypropyl group in CP-GS may arise from a intermediate metabolite of valine, such as methacrylic acid or its coenzyme A ester. It was also reported that the administration of CP-GS to rabbit resulted in the appearance of CP-Cys and N-acetyl-S-(2-carboxypropyl)cysteine in urine as metabolic products of CP-GS (Suzuki et al., 1962b). The biochemical significance of CP-GS in plants is not elucidated yet. We have made a HPLC determination of CP-GS in vegetables of Liliflorae (onion, garlic, narcissus, yellow leek, green leek, wakegi, spring onion) and then have tried to understand the biological role of CP-GS.



CP-GS

MATERIALS AND METHODS

Reagents. 2,4-Dinitrofluorobenzene (DNB) was purchased from Katayama Chemical Industries Co. (Osaka, Japan). Acetonitrile and methanol for HPLC were obtained from Wako Pure Chemical Industries (Osaka, Japan). Methacrylic acid was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Optically active methyl L-(S)- and methyl L-(R)-β-hydroxyisobutyrate and glutathione were supplied by Kanegafuchi Chemical Industry Co., Ltd. (Hyogo, Japan), and Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan), respectively. CP-Cys was synthesized in our laboratory (Ohmori et al., 1965).

Synthesis of CP-GS. Methacrylic acid (1.0 g) and glutathione (1.2 g) were dissolved in 12 mL of water, and the resultant solution was neutralized with $NaHCO_3$ and allowed to stand for 24 h at room temperature. After the reaction mixture was acidified with 1 M HCl and extracted with ether, it was applied to a Diaion SK (H^+ type) column (15 × 2 cm) and eluted with 2 M NH_3. The ammonia eluate was then evaporated to dryness under vacuum. The residue was dissolved in 5 mL of water and the solution was put on a DEAE Sephadex 25 column (41 × 3 cm) previously prepared by washing with 600 mL of 1 M acetic acid. The column was washed with 1.87 L of 1 M acetic acid and then fractionated with 1 M acetic acid. The eluates were tested by paper chromatography. The R_f value of CP-GS was 0.31, and that of Glu was 0.20 (n-BuOH:AcOH:water = 4:1:1). The fractions containing CP-GS were combined and dried under reduced pressure. The residue was recrystallized from water and alcohol; yield 500 mg. Anal. Calcd for $C_{14}H_{23}N_3O_8S$: C, 42.74; H, 5.88; N, 10.68. Found: C, 42.56; H, 5.87; N, 10.91. The peptide was also prepared from glutathione and β-bromoisobutyric acid by Virtanen and Matikkala (1960). The mass spectrum were taken with a Shimadzu GCMS 9020-DF instrument at 70 eV. The fast atom bombardment mass spectrum of the peptide showed a parent peak at m/z 394 $(M + H)^+$, which agreed with the molecular weight of CP-GS.

HPLC. A Shimadzu liquid chromatography, Model 3A (Kyoto, Japan), equipped with a Shimadzu variable-wavelength detector and a Shimadzu SGR-1A step gradient apparatus was used for determination of CP-GS. A 150 × 4.6 mm (i.d.) Inertsil ODS column (particle size 5 μm; Gaschro-kogyo, Osaka, Japan) with isocratic elution using a mixture of 10 mM potassium dihydrogen phosphate adjusted to pH 2.1 with phosphoric acid and acetonitrile (80:20, v/v) was used. The flow rate was 1.0 mL/min. After each analysis the column was washed by methanol/water (70 + 30 by volume) for 5 min and equilibrated with the elution buffer for 20 min by a program. All runs were performed at 44 °C, and the eluate was monitored at 380 nm.

Extraction of CP-GS from Vegetables. All vegetables, onion (*Allium cepa* L.), garlic (*Allium sative* L. cv. *nipponicum*), narcissus (*Narcissus tazetta* L. *var. chinensis* Roem.), yellow leek (*Allium* tuberosum Rottl.), green leek (*Allium tuberosum* Rottl.), wakegi (*Allium fistolosum* L. var. *caespitosum* Makino.), and spring onion (*Allium fistulosum L.* var. *giganteum* Makino.), were purchased from appropriate local stores. They were homogenized in 3 volumes of water at 0 °C for 2 min with use of a Potter-Elvehjem homogenizer with a Teflon pestle or a stainless grater. The homogenate was strained through cheesecloth. A 1-mL aliquot was mixed with 2 mL of methanol on a Vortex mixer and centrifuged at 1000g for 15 min. The supernatant was transferred to a 20-mL vial. The precipitate was homogenized with 1 mL of methanol/water (70 + 30 by volume) and centrifuged as mentioned above. This procedure was repeated again. The methanol extracts thus obtained were combined and evaporated on a rotary evaporator at 30 °C.

DNP Derivatization of CP-GS. After evaporation of the methanol extract, the residue was dissolved in 1 mL of 40 mM acetate buffer (pH 5.0). This solution was placed onto a QAE Sephadex A-25 column (2 mL) previously equilibrated with 40 mM acetate buffer (pH 5.0). The column was washed successively with 5 mL of 40 mM acetate buffer (pH 5.0) and 2.5% acetic acid. CP-GS was then eluted with 5 mL of 10% acetic acid. The eluate was placed in a 5-mL vial and evaporated with the Speed Vac concentrator (Model SVC-100H). To this vial were added 200 μL of 10% sodium carbonate solution and 10 μL of DNB. The vial was tightly capped and shaken mechanically at room temperature for 1 h. After reaction, 80 μL of 6 M HCl was added to the reaction mixture, and it was extracted for 30 s with 1 mL of benzene on a Vortex mixer. A 10-μL aliquot of the aqueous phase was subjected to HPLC. Typical chromatograms of authentic and onion DNP-CP-GS are shown in parts a and b of Figure 1, respectively. In Figure 1a, an aliquot (20 μL) of CP-GS aqueous solution (1 mg/mL) was placed in a 5-mL vial, followed by evaporation under a nitrogen stream. CP-GS in the dried

Faculty of Pharmaceutical Sciences, Okayama University, Tsushima-Naka-1, Okayama 700, Japan.

0021-8561/89/1437-0611$01.50/0

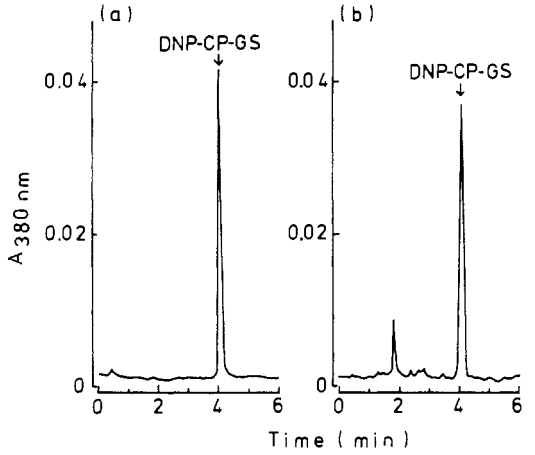


Figure 1. High-performance liquid chromatograms of DNP derivatives of authentic CP-GS (a) and that obtained from onion (b).

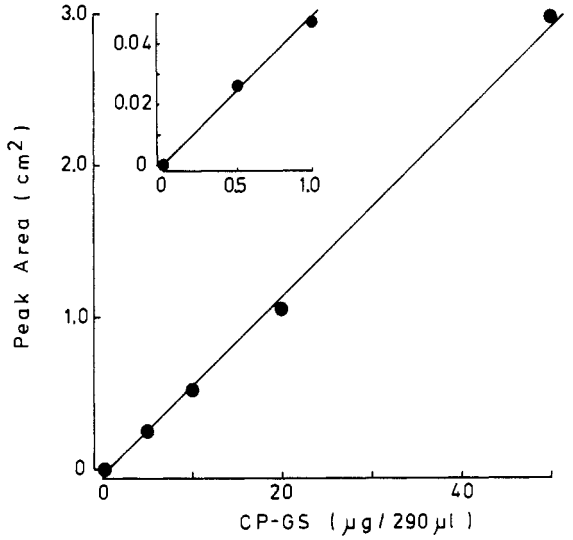


Figure 2. Calibration curve of CP-GS.

residue was reacted with DNB and subjected to HPLC as described above. In Figure 1b, onion (1 g) was treated with methanol and DNB as described above. A 10-μL aliquot of the aqueous phase (290 μL) was injected into HPLC.

Calibration Curve of CP-GS. Authentic CP-GS (1 mg) was dissolved in 1 mL of H_2O, and 0.5, 1.0, 5.0, 10.0, 20.0, and 50.0 μL of that solution was reacted with DNB. After reaction, it was acidified with 6 M HCl and extracted with benzene to remove unreacted DNB. A 10-μL aliquot of the aqueous layer was analyzed by HPLC.

Contents and Distribution of CP-GS in Various Vegetables. Vegetable tissues were homogenized in water, and the homogenates were centrifuged. After deproteinization of the supernatants, CP-GS was partially purified through a QAE Sephadex column and then derivatized by DNB in sodium carbonate solution. The reaction mixture was acidified by HCl and extracted with benzene. The aqueous layer was analyzed for DNP-CP-GS by HPLC.

RESULTS

Calibration Curve. A calibration curve for CP-GS is shown in Figure 2, indicating good linearity. The useful detection limit was 44 pmol by the present method.

Recovery Test. Various amounts of CP-GS were added to onion homogenate, which was treated by the procedure described in Extraction of CP-GS from Vegetables. The results are summarized in Table I and show a recovery of 91.3 ± 3.8%. As a separate test, 0.5, 1.0, and 2.0 μg of CP-GS was added to spring onion homogenate; the recovery was 85.8 ± 4.83%. The recoveries of CP-GS from other vegetables were also satisfactory.

Table I. Recovery of CP-GS Added to Onion Homogenate[a]

CP-GS added, μg/0.5 g	recovery: CP-GS determined, μg/0.5 g	%	CV, %	mean ± SD
0	55.36			91.3 ± 3.8
10	64.84	94.8	2.1	
20	73.95	93.0	4.1	
30	81.15	86.0	6.2	

[a] Various amounts of CP-GS were added to the onion homogenate that was treated by the procedure described in the text. Each value is the mean of triplicate determinations.

Table II. Contents and Distribution of CP-GS in Various Vegetables

	CP-GS, μg/g
onion	125
garlic	92.5
narcissus	ND[a]
yellow leek	0.64
green leek	1.23
wakegi	3.03
spring onion	0.61

[a] ND = not detectable.

Table III. Distribution of CP-GS in a Spring Onion

onion part[a]	CP-GS, μg/g
upper green stem	ND[b]
lower green stem	0.38
white part	0.75
root	1.3

[a] A spring onion was divided into four parts: green leaves, upper part of green stem, lower part of green stem, and white part of stem. CP-GS in each part was analyzed as described in Figure 2. [b] ND = not detectable.

Contents and Distribution of CP-GS in Various Vegetables of Liliflorae. The present method was applied to determination of CP-GS in various vegetables. As listed in Table II, CP-GS was found in most Liliflorae, especially in onion in a considerable amount. We examined distribution of CP-GS in five parts of a spring onion. As listed in Table III, CP-GS was distributed mostly in the root of a spring onion.

Identification of the Peak of the DNP Derivative of CP-GS from Onion on the HPLC. Identification of the DNP derivative of CP-GS from onion was performed as follows: The DNP-CP-GS derivative was synthesized from authentic CP-GS, hydrolyzed by 6 M hydrochloric acid at 105 °C for 24 h, and reacted again with DNB. Analyses of the DNP derivatives of amino acids by HPLC gave four peaks with retention times of 6.4, 8.4, 15.2, and 16.4 min, corresponding to the derivatives of L-glutamic acid, glycine, and the two optical isomers of CP-Cys, respectively. The ratio of peak areas was 9.1:1.4:1.2:1. In comparison, DNP derivatives of amino acids from the DNP derivative of CP-GS from onion also gave the four peaks with a peak area ratio of 9.7:1.3:1.1:1. Since DNP-CP-Cys has two diastereomeric relationships, it gave different elution times. If the reaction rate of the hydrolysis of the peptide and that of the derivatization of amino acids by DNB are the same, and, furthermore, the DNP derivatives of amino acids are not extracted by benzene and have the same extinction coefficient at 380 nm, no difference between peak areas of the DNP derivatives of amino acids must be observed. In a previous paper, the DNP derivatives of *S*-(1,2-dicarboxyethyl)glutathione were

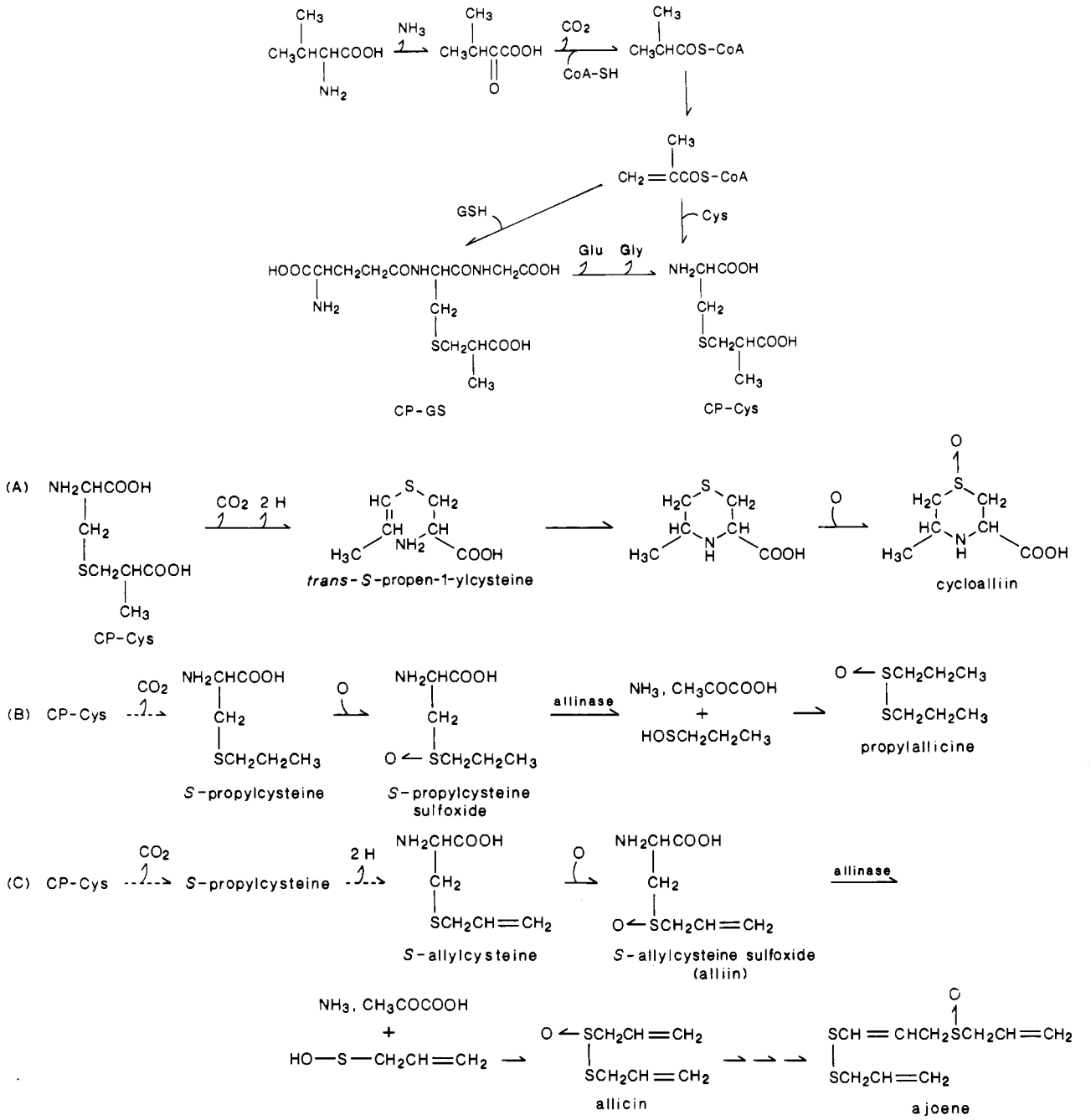


Figure 3. (Top) Biosynthetic pathways of CP-GS and CP-Cys. (Bottom) Metabolic pathways of CP-GS. Key: solid arrow, reported pathway; broken arrow, expected pathway; GSH, glutathione; Cys, L-cysteine.

hydrolyzed in 6 M HCl at 105 °C for 9 h. After the hydrolysate was dried in vacuo, it was reacted again with DNB. Analysis of the DNP derivatives by HPLC gave four peaks from two diastereomers of *S*-(1,2-dicarboxyethyl)-L-cysteine, glycine, and glutamic acid with peak area ratio 1.1:1:2:8.7 (Tsuboi et al., 1984). For these reasons, it can be concluded that the peak corresponding to DNP-CP-GS from the onion homogenate on HPLC was genuine and had no contamination.

DISCUSSION

Since CP-GS was isolated from ion and garlic (Virtanen and Matikkala, 1960; Suzuki et al., 1961a), no analytical procedure for this peptide has been established. In this report a sensitive and specific determination of CP-GS using HPLC was devised. We applied the procedure to determination of CP-GS in various vegetables of Liliflorae. As shown in Table II, it was found in onion in an especially high concentration and other vegetables also contained considerable amounts of CP-GS. Because of relatively high concentrations of CP-GS, it seems to have the same physiological role in those vegetables.

We show here the biosynthetic pathways of CP-GS and CP-Cys and the metabolic pathways of CP-Cys in Figure 3 because they have not been reviewed and will used for elucidation of this report. It was reported in garlic that CP-GS and CP-Cys were synthesized by the addition reaction of the SH group of glutathione or cysteine with the α,β-double bond of the methacrylic acid or methacrylyl-CoA formed from valine (Suzuki et al., 1962a). In 1960 Virtanen and Matikkala reported that CP-Cys was also present in onion. CP-Cys in onion appears to be directly formed from cysteine as in garlic. However, this route is relatively minor and CP-Cys is mainly formed via CP-GS, since the amount of free cysteine was small in onion (Granroth and Virtanen, 1967).

In the bottom part of Figure 3, we illustrate three different metabolic pathways of CP-Cys. Granroth and Virtanen reported pathway A in 1967 that CP-Cys was metabolyzed via *trans*-*S*-propen-1-ylcysteine in onion to cycloalliin, which was present in the vegetables of the *Allium* species (*A. cepa*, *A. sativum*, *Allium porrum*); its highest content was found in onion imported from Hungary (Virtanen and Matikkala, 1959).

In pathway B, *S*-propylcysteine and *S*-propylcysteine sulfoxide were found in onion (Virtanen and Matikkala, 1959a). *S*-Propylcysteine is converted to *S*-propylcysteine sulfoxide by oxidation (Virtanen and Matikkala, 1959b) and metabolized to pyruvate, ammonia, and propylallicin, present in vegetables of *Allium* species, by allinase (Schwimmer et al., 1960; Fujiwara et al., 1955).

In pathway C, *S*-allylcysteine (Renis and Henze, 1958), a precursor of *S*-allylcysteine sulfoxide (alliin; Suzuki et al., 1961b), is converted to alliin. Alliin is present mainly in the vegetables of *Allium* species and is degraded on crushing the vegetables into *S*-allyl 2-propenethiosulfinate (allicin) by the action of allinase, which was found only in the vegetables of *Allium* species (Fujiwara et al., 1958). It was reported that allicin was isolated from garlic and had antibacterial activity (Cavallito and Bailey, 1944) and was generally present in the vegetables of *Allium* species (Fujiwara et al., 1955). Ajoene, (*E*,*Z*)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide, which is formed from allicin, a potent antiplatelet compound obtained from garlic (Apitz-Castro et al., 1983; Block et al., 1984), inhibits platelet aggregation induced in vitro by ADP, collagen, epinephrine, thrombin, arachidonic acid, and platelet aggregation factors (Apitz-Castro et al., 1983). The antiplatelet action has been studied at physiological, pharmacological, and biochemical levels (Apitz-Castro et al., 1986; Newman et al., 1982).

We assumed that CP-GS was metabolized to ajoene by pathway C during storage or on injuring vegetables tissues. In order to elucidate this hypothesis, changes in CP-GS amounts were examined during storage and on injuring vegetables tissues. A grated onion was kept at room temperature for 2 days in September, and CP-GS contents were determined. Unexpectedly, no significant change in CP-GS contents was observed before and after storage. In onion and garlic the CP-GS contents were determined in summer (September) and in winter (February). We also found no changes of CP-GS contents in the both foods. Consequently, CP-GS was not metabolized during storage and on injuring vegetables tissues by these pathways.

We then examined the distribution of CP-GS in a spring onion (Table III). CP-GS was found mostly in the root of this vegetable, and a decrease in the content of CP-GS was observed with increasing distances from root. The distribution of CP-GS in a leek (*A. tuberosum* Rottl.) and a wakegi (*A. fistulosum* L. var. *caespitosum* Makino.) was similar to that in a spring onion (*A. fistulosum* L. var. *giganteum* Makino, data not shown). We are now examining why CP-GS is found mostly in the root.

We also studied the biosynthesis of CP-GS in onion homogenate. Suzuki et al. (1962b) reported that the origin of the 2-carboxypropyl group of CP-GS was assumed to be an intermediate metabolite of valine. In onion homogenate we examined the biosynthesis of CP-GS in four kinds of reaction systems. The first system consisted of glutathione and L-Val, the second L-Glu, L-Cys, Gly, L-Val, and ATP, and the third L-Glu, CP-Cys, Gly, and ATP as substrate. Substrates were incubated in 50 mM potassium phosphate buffer (pH 7.5) at 25 °C for 1 h. In all systems, however, no formation of CP-GS was observed. The first system was demonstrated in garlic by Suzuki et al. to be a pathway in CP-GS biosynthesis in vitro. Recently, we reported the enzyme that catalyzed dehydration from alcohol and thiol was present in yeast and rat tissues (Tsuboi and Ohmori, 1987). Hence, as the fourth system, glutathione and L-(*S*)- and L-(*R*)-β-hydroxyisobutyric acids were used as substrates in onion homogenate and reacted at 25 °C for 1 h, but CP-GS was not detected. From these results it appears that another biosynthesis pathway of CP-GS, other than those mentioned above, may exist in onion bulb.

ACKNOWLEDGMENT

We thank the Mishima Kaiun Foundation for support of this work.

Registry No. CP-GS, 6710-22-1; methacrylic acid, 79-41-4; glutathione, 70-18-8.

LITERATURE CITED

Apitz-Castro, R.; Cabrera, S.; Cruz, M. R.; Ledezma, E.; Jain, M. K. Effects of Garlic Extract and Three Pure Components isolated from it on Human Platelet Aggregation, Arachidonate Metabolism, Release Reaction and Platelet Ultrastructure. *Thrombosis Res.* **1983**, *32*, 155–169.

Apitz-Castro, R.; Ledezma, E.; Escalante, J.; Jain, M. K. The Molecular Basis of The Antiplatelet Action of Ajoene: Direct Interaction with The Fibrinogen Receptor. *Biochem. Biophys. Res. Commun.* **1986a**, *141* (1), 145–150.

Apitz-Castro, R.; Escalante, J.; Vargas, R.; Jain, M. K. Ajoene, The Antiplatelet Princeple of Garlic, Synergistically Potentiates The Antiaggregatory Action of Prostacyclin, Forskolin, Indomethacin and Dypiridamole on Human Platelets. *Thrombosis Res.* **1986b**, *42*, 303–311.

Block, E.; Ahmad, S.; Jain, M. K.; Crecely, R. W.; Apitz-Castro, R.; Cruz, M. R. (E,Z)-Ajoene: A Potent Antithrombotic Agent from Garlic. *J. Am. Chem. Soc.* **1984**, *106*, 8295–8296.

Cavallito, C. J.; Bailey, J. H. Allicin, the Antibacterial Principle of *Allium sativum*. 1. Isolation, Physical Properties and Antibacterial Action. *J. Am. Chem. Soc.* **1944**, *66*, 1950–1951.

Fujiwara, M.; Yoshimura, M.; Tsuno, S. Allithiamine, A Newly found Derivative of Vitamin B_1; III. on the Allcin Homologues in the Plants of the Allium Species. *J. Biochem.* **1955**, *42* (2), 591–601.

Fujiwara, M.; Yoshimura, M.; Tsuno, S.; Murakami, F. Allithiamine, A Newly found Derivative of Vitamin B_1; IV. on the Alliin Homologues in the Vegetables. *J. Biochem.* **1958**, *45* (3), 141–149.

Granroth, B.; Virtanen A. I. S-(2-Carboxypropyl)-cysteine and its Sulfoxide as Precursors in the Biosynthesis of Cycloalliin. *Acta Chem. Scand.* **1967**, *21*, 1654–1680.

Mizuhara, S.; Ohmori, S. A New Sulfur-Containing Amino Acid. *Arch. Biochem. Biophys.* **1961**, *92*, 53–57.

Newman, P. J.; Knipp, M. A.; Kahn, R. A. Extraction and Identification of Human Platelet Integral Membrane Proteins using Triton X-114. *Thrombosis Res.* **1982**, *27*, 221–224.

Ohmori, S.; Shimomura, T.; Azumi, T.; Mizuhara, S. S-(β-Carboxy-n-propyl)-L-cysteine and S-(β-Carboxyethyl)-L-cysteine in urine. *Biochem. Z.* **1965**, *343*, 9–15.

Renis, H. E.; Henze, R. E. Cysteine Derivatives in Mature Onion Bulbs. *Food Res.* **1958**, *23*, 345–350.

Schwimmer, S.; Carson, J. F.; Makower, R. U.; Mazelis, M.; Wong, F. F. Demonstration of Allinase in a Protein Preparation from Onion. *Experientia* **1980**, *16*, 449–450.

Suzuki, T.; Sugii, M.; Kakimoto, T. Newly γ-Glutamyl Peptides in Garlic. *Chem. Pharm. Bull.* **1961a**, *9*, 77–78.

Suzuki, T.; Sugii, M.; Kakimoto, T.; Tsuboi, N. Isolation of (-)-S-Allyl-L-Cysteine from Garlic. *Chem. Pharm. Bull.* **1961b**, *9*, 251–252.

Suzuki, T.; Sugii, M.; Kakimoto, T. Metabolic Incorporation of L-Valin-[^{14}C] into S-(2-Carboxypropyl)glutathione and S-(2-Carboxypropyl)cysteine in Garlic. *Chem. Pharm. Bull.* **1962a**, *10*, 328–331.

Suzuki, T.; Sugii, M.; Kakimoto, T. Metabolism of S-(2-Carboxypropyl)glutathione in Rabbit. *Chem. Pharm. Bull.* **1962b**, *10*, 346–347.

Tsuboi, S.; Ohmori, S. *SEIKAQ* **1987**, *59* (8), 895.
Tsuboi, S.; Uda, N.; Ikeda, M.; Hirota, K.; Ohmori, S. S-(1,2-Dicarboxyethyl)glutathion and S-(1,2-Dicarboxyethyl)L-cysteine in Lens. *J. Clin. Chem. Clin. Biochem.* **1984**, *22*, 285–290.
Virtanen, A. I.; Matikkala, E. J. The Structure and Synthesis of Cycloalliin Isolated from *Allium cepa*. *Acta Chem. Scand.* **1959a**, *13* (3), 623–626.
Virtanen, A. I.; Matikkala, E. J. The Isolation of S-Methyl-cysteinesulphoxide and S-n-Propylcysteinesulphoxide from Onion (*Allium cepa*) and the Antibiotic Activity of Crushed onion. *Acta Chem. Scand.* **1959b**, *13*, 1898–1900.
Virtanen, A. I.; Matikkala, E. J. Neue γ-Glutamylpeptide in Zwiebel (*Allium cepa*). *Hoppe-Seyler's Z. Physiol. Chem.* **1960**, *322*, 8–20.

Received for review January 5, 1988. Revised manuscript received June 13, 1988. Accepted September 26, 1988.

Use of Polyamide Oxidative Fluorescence Test on Lipid Emulsions: Contrast in Relative Effectiveness of Antioxidants in Bulk Versus Dispersed Systems[1]

William L. Porter,* Edward D. Black, and Anne M. Drolet

The polyamide fluorescence method of detecting malonaldehyde or precursors has been adapted to wet systems, using liposomes from sonicated soybean lecithin. Vapors arising from the oxidizing microdispersions produce fluorescent compounds with the end-group amines contained in a polyamide powder coated on plastic or glass. We have studied the hematin-accelerated reaction in covered Petri dishes with a polyamide strip in the vapor space at a temperature of 65 °C, using 25 mL of 0.013 M phosphate buffer, pH 5.5, containing 3 mg/mL sonicated lecithin. At pH 9.1 no fluorescence is measurable. The plate fluorescence intensity is measured with a solid sample holder in a fluorescence spectrophotometer. There is an excitation maximum at 360 nm and an initial emission maximum at 425 nm, shifting bathochromically with time. A series of antioxidants tested in the hematin-catalyzed system at 0.1% phospholipid display a largely reciprocal relation to their reported effectiveness in dry bulk oils, particularly vegetable oils. Further evidence for this "polar paradox" that nonpolar antioxidants function best in polar lipid emulsions and membranes while polar antioxidants are relatively more effective on nonpolar lipids is presented.

The military experience with lipids in foods has a wide range: storage of multipurpose cooking and salad oils, frying with these oils, storage and use in mayonnaise and salad dressing emulsions, comminuted and re-formed meats, baked goods, and freeze-dried items, to mention only a few. Oxidation of lipids, whether initiated by enzymatic, photooxidative, or metal catalysis, is a universal problem in shelf life of military rations. Antioxidants introduced to prevent or delay this process must be used in a wide variety of situations. Experience has shown that antioxidants' relative effectiveness varies widely. No general rationale for their use has been developed. Availability of such a protocol would result in large extensions of shelf life of military rations.

Porter has suggested (1980) that a useful rationale to simplify the many applications of primary antioxidants is that, ceteris paribus, compounds that are relatively polar, hydrophilic, or amphiphilic with high hydrophile–lipophile balance (HLB) number (Griffith, 1954; Adamson 1967) are relatively more effective in low surface to volume ratio (LSV) displays of lipid (bulk oils and fats, whether vegetable or animal, or synthetic esters of their constituent fatty acids). On the other hand, compounds that are relatively nonpolar, lipophilic, or amphiphilic with low HLB are relatively more effective in high surface to volume (HSV) lipid situations (emulsions, micelles, and membranes of whole tissue having colloidal dimensions, whether hydrated or dehydrated). The proposed rule is most applicable at the extremes of a continuum, i.e., in emulsions and membranes with a very low lipid phase concentration versus bulk vegetable oils. However, the proposed rule also generally applies to bulk animal fats and oils and to synthetic esters derived from these as well as to concentrated emulsions. Clearly, high volatility (BHA, BHT) or vulnerability to heat and alkaline conditions, as in the short-chain substituted hydroquinones and gallates, modifies the general trend, as do the introduction and processing conditions that emphasize these traits. Endogenous tocopherols also modify the effect in bulk vegetable oils.

Before 1955, applications of antioxidants and the tests for their relative effectiveness in stabilizing lipids were largely with dry bulk (LSV) fats and oils. Stability tests like the AOM test, the Schaal oven test, and the oxygen bomb test were most adaptable to bulk lipid, whether monitored by peroxide value (PV), pressure change, weight gain, or sensory change. A large body of relative effectiveness data for antioxidants was thus generated, and very often it has been extrapolated uncritically from bulk lipid (LSV) to HSV situations like emulsions, micelles, and

Department of the Army, U.S. Army Troop Support Command, Natick Research, Development & Engineering Center, Natick, Massachusetts 01760-5020.

[1] Presented at the meeting of the Division of Agricultural and Food Chemistry, 182nd National Meeting of the American Chemical Society, New York, NY, 1981.