Selective oxidation of histidine residues in proteins or peptides through the copper(II)-catalysed autoxidation of glucosone

Rong-Zhu CHENG, Koji UCHIDA and Shunro KAWAKISHI*

Department of Food Science and Technology, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-01, Japan

Glucosone has been identified as the main intermediate sugar moiety product of the copper(II)-catalysed autoxidation of the Amadori compound [Kawakishi, Tsunehiro & Uchida (1991) *Carbohydr. Res.* **211**, 167–171]. Oxidative fragmentation of the model protein, especially selective degradation of the histidine residue in protein or peptides mediated by the copper(II)-catalysed autoxidation of glucosone, is discussed in this paper. The oxidative damage to protein could be retarded by catalase (EC 1.11.1.16) and EDTA, while superoxide dismutase (EC 1.15.1.1) and hydroxyl radical scavengers showed little effect. Through the process of the oxidative degradation of *N*-benzoylhistidine and other histidine-containing peptides, the oxidation of the imidazole ring in histidine caused by the glucosone–copper(II) system was the same as that by the ascorbate–copper(II) system. These facts suggest that the copper-catalysed autoxidation of glucosone could generate some active-oxygen species causing oxidative damage to protein similar to that caused by the ascorbate–copper(II) system.

INTRODUCTION

Glycation, also known as nonenzymic glycosylation, is initiated by the condensation of a glucose molecule with a free amino group of a protein. It is assumed to proceed via a Schiff's base intermediate, followed by Amadori rearrangement to yield relatively stable ketoamine adducts of proteins (Isbell & Frush, 1958; Gottschalk, 1972). When glycation was detected as a common post-translational modification of other proteins *in vivo*, it was quickly recognized that non-enzymic glycation of protein might promote several diseases associated with diabetes (Cerami *et al.*, 1979; Bunn, 1981; Brownlee *et al.*, 1984, 1988), aging (Chiou *et al.*, 1981; Monnier & Cerami, 1981; Kohn & Schnider, 1982), and cataracts (Pirie, 1968; Ortwerth *et al.*, 1988).

In recent studies on the metal ion-catalysed oxidation of glycated protein (Cheng *et al.*, 1991), the generation of α -dicarbonyl compounds and selective degradation of histidine residues in protein have been observed. Moreover, glucosone, one kind of α -dicarbonyl compound, has also been identified as a main sugar moiety product during the metal ion-mediated autoxidation of the Amadori compound (Kawakishi *et al.*, 1991) as well as glycated peptides (results not shown), although 3-deoxyglucosone (Kato, 1962), *N*-(carboxymethyl)lysine (CML) and 3-(*N*-lysino)lactic acid (Ahmed *et al.*, 1986, 1988; Baynes, 1991) have been identified during the process of degradation of the Amadori compound.

On the other hand, it has been reported that in the early steps of non-enzymic glycation of proteins, superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) are generated (Sakurai & Tsuchiya, 1988; Cathleen *et al.*, 1990; Jiang *et al.*, 1990) and through the metal ion-catalysed autoxidation of the Amadori compound, lipid peroxidation (Sakurai *et al.*, 1990, 1991), protein damage (Kawakishi *et al.*, 1990) and cleavage of nucleic acid (Kashimura *et al.*, 1986) occur. This oxidative damage might be caused by reactive oxygen radicals, but the reaction mechanism is still not clearly understood.

To clarify the oxidation process of glycated protein mediated by metal ions, glucosone was used as the active mediator in the

metal-catalysed oxidation, and then the oxidative fragmentation of proteins and the selective oxidation of the imidazole ring in the histidine derivatives were characterized.

MATERIALS AND METHODS

Reagents

BSA was purchased from Seikagaku Kogyo Co. Ltd. (Tokyo, Japan). Catalase (EC 1.11.1.16) (from bovine liver), superoxide dismutase (SOD; EC 1.15.1.1) (from bovine erythrocytes), L-histidyl-L-tyrosine, and *N*-benzoylhistidine were obtained from Sigma Chemical Co. β -Alanine-L-histidine was purchased from the Peptide Institute, Inc. (Osaka, Japan). D-Glucosone was prepared by the procedure of Bayne (1963). All other reagents were of the highest grades commercially available.

Reaction of BSA or histidine-containing peptides with glucosone/copper(II)

The reaction mixtures (4 ml) containing 0.04% (w/v) BSA or 1 mM peptides, 5 mM-glucosone and 0.05 mM- $CuSO_4$ in 67 mM-phosphate buffer, pH 7.2, were incubated at 37 °C. The reaction was initiated by the addition of glucosone and stopped by the addition of EDTA solution (0.1 mM).

SDS/PAGE

SDS/PAGE using 10% acrylamide gel was performed by the method of Laemmli (1970). The gel sheet was stained with a solution of 0.25% (w/v) Coomassie Brilliant Blue R-250 in water/propan-2-ol/acetic acid (5:5:1, by vol.) and destained with 7% (v/v) acetic acid containing 5% (v/v) methanol.

Amino acid analysis

Amino acid analysis was performed with a JEOL JLC-300 amino acid analyser, for which the sample was prepared as follows. The reaction mixtures containing 0.04% (w/v) BSA, 5 mM-glucosone and 0.05 mM- Cu^{2+} in 67 mM-phosphate buffer, pH 7.2, were treated with 6% (w/v) trichloroacetic acid solution, and the precipitated protein was hydrolysed in a sealed tube with 6 M-HCl at 110 °C for 24 h. The hydrolysates were concentrated

Abbreviations used: CML, *N*-(carboxymethyl)lysine; DMSO, dimethyl sulphoxide; SOD, superoxide dismutase; 3-DG, 3-deoxyglucosone; TFA, trifluoroacetic acid.

* To whom correspondence and reprint requests should be addressed.

to dryness, dissolved in dil. HCl solution (pH 2.2), and then placed in the amino acid analyser.

H.p.l.c.

The peptide was determined by reversed-phase h.p.l.c. on a Develosil ODS-5 column (4.6 mm × 250 mm). Chromatographic conditions were as follows. Samples were eluted at a rate of 0.8 ml/min with 0.05 M-ammonium acetate/methanol (97:3, by vol.) for histidyltyrosine, and 0.05 M-ammonium acetate/methanol (75:25, by vol.) for benzoylhistidine, and at a rate of 0.5 ml/min with 0.05 M-ammonium acetate/methanol (99:1, by vol.) for β -alanylhistidine. The eluates were monitored at 210 nm. Areas under the chromatographic peaks for each material were calculated using a Shimadzu Chromatopac C-R 3A integrator. Loss of histidine residue in each peptide was also determined by amino acid analysis, for which the samples were prepared as follows. After the mixtures were freeze-dried, they were hydrolysed with 6 M-HCl at 110 °C for 24 h. The hydrolysates were then concentrated, dissolved in aq. HCl (pH 2.2), and submitted for analysis.

RESULTS

Oxidative damage of BSA with glucosone-copper(II) system

Time-dependent alterations of BSA treated with glucosone-copper(II) were characterized by SDS/PAGE as shown in Fig. 1. In the presence of copper(II), the major band of the native BSA (67 kDa) almost disappeared within 4 h of incubation, and oxidized lower-molecular-mass fragments (below 14 kDa) appeared. High-molecular-mass bands (above 67 kDa) were not observed. However, no change in BSA was observed in the absence of copper(II). In addition, we also confirmed significant fragmentation of BSA during incubation with glucosone-copper(II) by h.p.l.c. on a TSK-GEL G 3000 SW column (results not shown).

Changes in the primary structure of protein during incubation with glucosone-copper(II) were determined by amino acid analysis. The results are represented as amino acid molar ratios (%) (Table 1). Since the samples for zero time and 24 h were always treated and hydrolysed under the same conditions, the remarkable changes in molar ratio between $t = 0$ and 24 h could represent the variation of amino acid residues, although the zero-time data are not equal to the theoretical data because of the different stability of amino acids during the process of hydrolysis by 6 M-HCl at 110 °C for 24 h. As shown in Table 1, a decrease in the histidine residue content was the most prominent change observed (83% decrease over 24 h). Moreover, decreases in lysine and cysteine residues and slight increases in aspartate and glutamate were also regarded as being significant. Arginine content appeared to be unchanged; the reason for this may be that copper(II)-catalysed autooxidation of glucosone is much faster (more than 60% of the glucosone was degraded within 8 h at 37 °C) than its reaction with arginine (results not shown). From these results it was shown that the glucosone-copper(II) system could cause both oxidative fragmentation of protein and degradation of histidine residues.

Glucosone, one kind of α -dicarbonyl compound, is very active chemically, as is ascorbate. Based on the results from many studies of the ascorbate-copper(II) system, we assumed that the oxidative reactions of proteins mediated by the glucosone-copper(II) system might arise from some oxygen radicals through an interaction between a glucosone-copper(II)-histidine complex and oxygen. A series of hydroxyl-radical scavengers, enzymes relating to the degradation of active oxygen, and chelating agents for metal ions were added to this system to characterize the inhibition effects. As shown in Table 2, only catalase and EDTA

completely depressed the oxidative fragmentation of BSA caused by the glucosone-copper(II) system, but the hydroxyl-radical scavengers [mannitol, dimethyl sulphoxide (DMSO), and urea] and SOD showed little effect on the oxidative reaction. This result is similar to that in the glycated protein-copper(II) system (Cheng *et al.*, 1991).

Selective damage to histidine-containing peptides

From the above results, the loss of histidine residue in BSA was the most prominent. In order to clarify whether this oxidative damage of histidine residues occurred selectively or not, a model

Fig. 1. SDS/PAGE of BSA treated with glucosone in the presence (b) and absence (a) of copper(II)

The reactions were carried out at 37 °C; the mixtures contained 0.04% (w/v) BSA, 5 mM-glucosone and 0.05 mM-CuSO₄ in 67 mM-phosphate buffer, pH 7.2. Each portion (0.5 ml) of the reaction mixture was sampled at 0, 4, 8, and 24 h and treated with 0.5 ml of trichloroacetic acid (12%, w/v) at 4 °C for 24 h, and the precipitates were submitted to SDS/PAGE.

Table 1. Changes in amino acid compositions of BSA through reaction with the glucosone-copper(II) system

The reaction was carried out at 37 °C for 24 h; the solutions contained 0.04% (w/v) BSA, 5 mM-glucosone and 0.05 mM-CuSO₄ in 67 mM-phosphate buffer, pH 7.2. Amino acid analysis was performed as described in the Materials and methods section. The molar ratio (%) represents the molar concentration of each amino acid as a percentage of the total amino acids.

Amino acid	Molar ratio (%)	
	0 h	24 h
Asp	8.8	9.9
Thr	5.0	5.5
Ser	3.8	4.4
Glu	12.6	14.4
Pro	4.6	4.7
Gly	2.8	3.0
Ala	7.1	7.9
Cys	3.3	2.6
Val	6.0	6.1
Met	0.8	0.6
Ile	2.3	2.2
Leu	10.0	10.3
Tyr	3.0	2.7
Phe	4.2	4.2
His	3.0	0.5
Lys	9.6	8.3
Arg	3.7	3.7

Table 2. Effects of several inhibitors on oxidative degradation of BSA with the glucosone-copper(II) system

The reactions were carried out at 37 °C for 24 h; the solutions contained 0.04 % (w/v) BSA, 5 mM-glucosone, 0.05 mM-CuSO₄ and inhibitor in 67 mM-phosphate buffer, pH 7.2. Oxidation of BSA was detected by h.p.l.c. on a TSK-GEL G3000SW column. Chromatographic conditions were as follows: column, TSK-GEL G3000SW (7.5 mm × 600 mm); eluate, 67 mM-phosphate buffer containing 100 mM-NaCl, pH 7.0; flow rate, 1.0 ml/min; detection, absorbance at 210 nm. All concentrations shown are final reaction concentrations. The percentage inhibition was calculated as follows:

$$\text{Inhibition (\%)} = 100 - [(A - X)/(A - B)] \times 100$$

where *A* is the peak height of BSA (0.04 %, w/v) in phosphate buffer after 24 h incubation, *B* is the peak height of BSA (0.04 %, w/v) after reaction with glucosone-copper(II) system for 24 h, and *X* is the peak height of the (*B* + inhibitor) system.

Inhibitor	Concentration	Inhibition (%)
None		0
EDTA	0.2 mM	97
Mannitol	10.0 mM	0
DMSO	10.0 mM	0
Urea	10.0 mM	0
Catalase	500 units/ml	74
SOD	500 units/ml	0

Table 3. Time-dependent changes in amino acid compositions of *N*-benzoylhistidine through reaction with glucosone-copper(II) system

Reactions were carried out at 37 °C; the solutions contained 1 mM-*N*-benzoylhistidine, 5 mM-glucosone and 0.05 mM-CuSO₄ in 67 mM-phosphate buffer, pH 7.2. The loss of histidine residue was determined by amino acid analysis as described in the Materials and methods section. Molar ratio (%) represents the molar concentration of each amino acid as a percentage of the total amino acids.

Amino acid	Molar ratio (%)				
	0 h	2 h	4 h	8 h	24 h
Asp	0	2.0	2.9	5.6	11.5
Gly	0	0.7	0.7	2.3	1.6
His	70.2	67.4	59.4	41.1	24.0
NH ₄ OH	29.8	27.8	37.7	41.1	63.0

Table 4. Time-dependent changes in amino acid compositions of *L*-histidyltyrosine through reaction with glucosone-copper(II) system

Conditions were as described in Table 3, except 1 mM-histidyltyrosine was used instead of *N*-benzoylhistidine.

Amino acid	Molar ratio (%)				
	0 h	2 h	4 h	8 h	24 h
Asp	0	0.2	0.3	0.3	0.5
Glu	0	0.3	0.4	0.5	0.8
Gly	0	0.4	0.5	0.7	1.2
Tyr	47.0	46.9	47.6	48.8	49.1
His	46.8	43.8	42.3	37.4	32.2
NH ₄ OH	6.1	9.3	9.8	13.7	18.7

reaction of the histidine-containing peptides with glucosone-copper(II) system was undertaken. Time-dependent degradation of histidine-containing peptides was characterized by h.p.l.c. as

Fig. 2. Time-dependent degradation of histidine-containing peptides during the reaction with glucosone-copper(II) system

The reactions were carried out at 37 °C; the mixtures contained 1 mM-peptide, 5 mM-glucosone and 0.05 mM-CuSO₄ in 67 mM-phosphate buffer, pH 7.2. The peptides were determined by reversed-phase h.p.l.c. as described in the Materials and methods section. ●, β-Alanylhistidine; ▲, L-histidyltyrosine; ■, *N*-benzoylhistidine; □, *N*-benzoyl-2-oxohistidine.

described in the Materials and methods section. As shown in Fig. 2, histidyltyrosine, β-alanylhistidine and *N*-benzoylhistidine were markedly degraded upon incubation. From amino acid analyses, their composition changes were determined as shown in Tables 3 and 4. Similar to the oxidation of proteins, only the histidine residue showed a marked decrease, and a considerable amount of ammonia was produced, while other amino acids showed no appreciable change.

In addition, for the purpose of establishing the oxidative degradation of histidine in this system, *N*-benzoylhistidine was chosen as histidyl residue analogue. The oxidation of *N*-benzoylhistidine mediated by either the glucosone-copper(II) system or the ascorbate-copper(II) system was investigated by reversed-phase h.p.l.c. using an ODS column, as shown in Fig. 3. The same oxidation pattern for *N*-benzoylhistidine was observed in both systems, and the main oxidation product, *N*-benzoyl-2-oxohistidine, was detected. On the other hand, the mechanism of metal ion-ascorbate-mediated specific oxidation of the imidazole ring in histidine derivatives has been studied by Uchida & Kawakishi (1989a,b, 1990a,b) in detail. It was found that the same oxidative degradation of the imidazole ring of histidine was caused not only by the ascorbate-copper(II) but also by the glucosone-copper(II) system. This result may be very useful for studying the mechanism of the metal ion-catalysed oxidation of glycated protein.

DISCUSSION

The non-enzymic reaction of glucose with protein is well known and is thought to be important in the aetiology of the long-term complications of diabetes (Harding, 1985). Wolff & Dean (1987) have proposed that in the transition-metal-catalysed autoxidation of glucose, α-ketoaldehyde (glucosone) and reactive oxygen radicals are formed. The α-ketoaldehyde is responsible for a significant proportion of the initial reaction of the non-enzymic glycosylation of protein and oxygen radicals which cause the oxidative damage to protein (Wolff *et al.*, 1984, 1991; Wolff & Dean, 1987; Hunt *et al.*, 1988). On the other hand,

Fig. 3. H.p.l.c. profile of the oxidation products of *N*-benzoylhistidine by reaction with ascorbate-copper(II) system (a) and glucosone-copper(II) system (b)

The reaction mixtures containing 1 mM-*N*-benzoylhistidine, 0.05 mM- CuSO_4 and 5 mM-ascorbate (a) or 5 mM-glucosone (b) in 67 mM-phosphate buffer, pH 7.2, were incubated at 37 °C for 24 h. Peaks 1 and 3 represent *N*-benzoylhistidine and *N*-benzoyl-2-oxohistidine respectively. Chromatographic conditions were as follows: column, Develosil ODS-5 (8 mm $\times$ 250 mm); eluate, water (0.1% TFA, v/v)/methanol (3:1, by vol.); flow rate, 1.5 ml/min; detection, absorbance at 230 nm.

Fig. 4. Proposed mechanism for the ring opening of the imidazole ring by glucosone-copper(II) system (Uchida & Kawakishi, 1989b)

Ahmed *et al.* (1986) have found that CML was generated from autoxidation of Amadori compound in phosphate buffer. However, we recently showed that, during the metal-mediated oxidation of glycated protein or Amadori compound, α -dicarbonyl compounds (Cheng *et al.*, 1991) or glucosone (Kawakishi *et al.*, 1991), not 3-deoxyglycosone (3-DG), was formed directly from Amadori compound. The discrepancy between our results and those of Ahmed might be due to whether or not transition metal ions had been added to the reaction system.

In cataracts, lenticular copper ion was found to be condensed, at concentrations 4–10-times higher than normal, in intact lenses (Shlopak, 1962, 1963, 1964; Nath *et al.*, 1969; Racz & Ordogh, 1977; Cook and McGahan, 1986). These results show that copper-ion-mediated oxidative damage to protein *in vivo*, especially that concerned with aging and cataracts, may be an important pathway which should be studied further. In our system, the α -dicarbonyl compounds were assumed to be very important intermediate products for causing the secondary oxidative damage to protein when this is catalysed by a metal ion. In this study, the oxidation of proteins, especially the selective degradation of histidine residues through the copper(II)-catalysed autoxidation of D-glucosone, has been investigated in detail.

As shown in Figs. 1 and 2, BSA or peptides were notably decomposed under the oxidative action of the glucosone-copper(II) system, but no changes were observed in the presence of glucosone only. Based on the amino acid analyses, the histidine residue of the protein (Table 1) and peptides (Table 3, Table 4) was selectively degraded by this oxidation system. These results indicated that the oxidation might be dependent upon some oxygen radicals or a histidine-copper-oxygen complex which caused site-specific damage to protein. To determine the radical species that participated in this oxidation, several inhibitors were applied to the BSA-glucosone-copper(II) system (Table 2). Among them, only EDTA and catalase completely inhibited this oxidation. This means that copper ion strongly participates in this reaction and the formation of hydrogen peroxide is required for the progress of this oxidation. However, the oxidation was not depressed upon the addition of either SOD or some radical scavengers, as shown in Table 2. These results are the same as those for the glycated protein-copper(II) system.

Fig. 3 shows that the same oxidative reactions occur in the imidazole ring of the histidine derivative with either the glucosone-copper ion or the ascorbate-copper ion system. Peak 3 represents the *N*-benzoyl-2-oxohistidine intermediate. Therefore, the selective oxidation of the imidazole ring in the histidine moiety through the metal-ion-catalysed autoxidation of glucosone may proceed by the same process as occurs with the ascorbate-copper ion system, as shown in Fig. 4. However, it seems that, in either the glycated protein-copper(II) system or the glucosone-copper(II)-protein system, the oxidative degradations of the imidazole ring in the histidine residue and the fragmentations of protein are not related to each other. Based on the experimental data, the main sites of fragmentation of the protein may be on some peptide bonds near the generation sites of the active oxygen radical or metal-ion-chelating sites, although such sites have not been identified. Furthermore, the present study will be significant for interpreting the selective oxidation of histidine residues in the copper(II)-catalysed oxidation of glycated proteins.

REFERENCES

- Ahmed, M. U., Thorpe, S. R. & Baynes, J. W. (1986) *J. Biol. Chem.* **261**, 4889–4994
- Ahmed, M. U., Dunn, J. A., Walla, M. D., Thorpe, S. R. & Baynes, J. W. (1988) *J. Biol. Chem.* **263**, 8816–8821

- Bayne, S. (1963) *Methods Carbohydr. Chem.* **2**, 421–423
- Baynes, J. W. (1991) *Diabetes*. **40**, 405–412
- Brownlee, M., Vlassara, H. & Cerami, A. (1984) *Am. Int. Med.* **101**, 527–537
- Brownlee, M., Cerami, A. & Vlassara, H. (1988) *N. Engl. J. Med.* **318**, 1315–1321
- Bunn, H. F. (1981) *Am. J. Med.* **70**, 325–330
- Cathleen, J. M., Edelstein, D. & Brownlee, M. (1990) *Biochem. Biophys. Res. Commun.* **173**, 932–939
- Cerami, A., Stevens, V. J. & Monnier, V. M. (1979) *Metabolism* **28**, 431–437
- Cheng, R. Z., Tsunehiro, J., Uchida, K. & Kawakishi, S. (1991) *Agric. Biol. Chem.* **55**, 1993–1998
- Chiou, S. H., Chylack, L. T., Jr., Tung, W. H. & Bunn, H. F. (1981) *J. Biol. Chem.* **256**, 5176–5180
- Cook, C. S. & McGahan, M. C. (1986) *Curr. Eye Res.* **5**(1), 69–76
- Gottschalk, A. (1972) *The Glycoproteins. Part A*, pp. 141–157, Elsevier, Amsterdam
- Harding, J. J. (1985) *Adv. Protein Chem.* **37**, 247–334
- Hunt, J. V., Dean, R. T. & Wolff, S. P. (1988) *Biochem. J.* **256**, 205–212
- Isbell, H. S. & Frush, H. (1958) *J. Org. Chem.* **23**, 1309–1319
- Jiang, Z., Woollard, A. C. S. & Wolff, S. P. (1990) *FEBS Lett.* **268**, 69–71
- Kashimura, N., Morita, J., Sato, J., Kumazawa, Z., Nisikawa, S., Ito, S., Koma, Y. & Komada, M. (1986) *Dev. Food Sci.* **13**, 401–410
- Kato, H. (1962) *Agric. Biol. Chem.* **26**, 187–192
- Kawakishi, S., Okawa, Y. & Uchida, K. (1990) *J. Agric. Food Chem.* **38**, 13–17
- Kawakishi, S., Tsunehiro, J. & Uchida, K. (1991) *Carbohydr. Res.* **211**, 167–171
- Kohn, R. R. & Schnider, S. L. (1982) *Diabetes* **31**, 47–51
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Monnier, V. M. & Cerami, A. (1981) *Science* **211**, 491–493
- Nath, R., Srivastava, S. K. & Singh, K. (1969) *Ind. J. Exp. Biol.* **7**, 25–26
- Ortwerth, B. J., Feather, M. S. & Olesen, P. R. (1988) *Exp. Eye Res.* **47**, 155–168
- Pirie, A. (1968) *Invest. Ophthalmol.* **7**, 634–650
- Racz, P. & Ordogh, M. (1977) *Albrecht von Graefes Arch. Klin. Exp. Ophthalmol.* **204**, 67–72
- Sakurai, T. & Tsuchiya, S. (1988) *FEBS Lett.* **236**, 406–410
- Sakurai, T., Sugioka, K. & Nakano, M. (1990) *Biochim. Biophys. Acta* **1043**, 27–33
- Sakurai, T., Kimura, S., Nakano, M. & Kimura, H. (1991) *Biochem. Biophys. Res. Commun.* **177**, 433–439
- Shlopak, T. V. (1962) *Oftalmol. Zh.* **17**, 347–351
- Shlopak, T. V. (1963) *Chem. Abstr.* **59**, 10618b
- Shlopak, T. V. (1964) *Chem. Abstr.* **60**, 9755b
- Uchida, K. & Kawakishi, S. (1989a) *J. Agric. Food Chem.* **37**, 897–910
- Uchida, K. & Kawakishi, S. (1989b) *Bioorg. Chem.* **17**, 330–343
- Uchida, K. & Kawakishi, S. (1990a) *J. Agric. Food Chem.* **38**, 660–664
- Uchida, K. & Kawakishi, S. (1990b) *J. Agric. Food Chem.* **38**, 1896–1899
- Wolff, S. P. & Dean, R. T. (1987) *Biochem. J.* **245**, 243–250
- Wolff, S. P., Crabbe, M. J. C. & Thornalley, P. J. (1984) *Experientia* **40**, 244–246
- Wolff, S. P., Jiang, Z. Y. & Hunt, J. V. (1991) *Free Radical Biol. Med.* **10**, 339–352

Received 10 September 1991/18 December 1991; accepted 8 January 1992