Mol. Nutr. Food Res. 2006, *50*, 725 – 731 DOI 10.1002/mnfr.200500250

Xanthine oxidase inhibitory activity of alkyl gallates

Noriyoshi Masuoka[1, 2], Ken-ichi Nihei[2] and Isao Kubo[2]

[1]Department of Life Science, Okayama University of Science, Ridai-cho, Okayama, Japan
[2]Department of Environmental Science, Policy and Management, University of California, Berkeley, California, USA

A series (C_1 – C_{12}) of alkyl gallates was examined for their effects on the activity of xanthine oxidase. Octyl (C_8), decyl (C_{10}), and dodecyl (C_{12}) gallates competitively inhibited uric acid formation generated by xanthine oxidase, and the inhibition increased upon increasing the alkyl chain length. Interestingly, neither menthyl nor bornyl gallates inhibited uric acid formation. These data indicate that the hydrophobic alkyl portion is associated with the xanthine-binding site in the Mo-binding domain. It is likely that the linear alkyl portion interacts with the hydrophobic domain close to the binding site, and the hydrophobic interaction is crucial to inhibit the xanthine oxidase reaction. On the other hand, all of gallic acid and its esters equally suppress superoxide anion generation catalyzed by xanthine oxidase at low concentration. The suppression is not due to scavenging activity of these gallates but due to reduction of xanthine oxidase by these gallates. The reduced enzyme catalyzes the reaction to generate hydrogen peroxide and uric acid.

Keywords: Alkyl gallates / Inhibition / Superoxide anion / Uric acid / Xanthine oxidase

Received: December 1, 2005; revised: March 25, 2006; accepted: March 28, 2006

1 Introduction

Xanthine oxidase (EC 1.1.3.22), a molybdenum-containing enzyme, catalyzes the oxidation of hypoxanthine to xanthine and finally to uric acid [1, 2]. With uric acid formation, xanthine oxidase reduces oxygen to superoxide anion and hydrogen peroxide. Accumulation of uric acid leads to hyperuricemia and gout [3, 4]. Hence, xanthine oxidase inhibitors could be useful as therapeutic agents for hyperuricemia and gout. In addition, superoxide anion generation by xanthine oxidase leads to peroxidative damages in cells [5], and the radical scavengers and the inhibitors are useful to prevent the postischemic tissue injury [6]. The xanthine oxidase reaction follows a ping-pong mechanism. With our study of antioxidant activity using xanthine oxidase reaction, we have become aware that the balance of hydrophilic and hydrophobic moieties of molecules is related to the inhibitory activity. For example, anacardic acid, 6-pentadecatrienylsalicylic acid (**1**) (Fig. 1), co-operatively inhibited uric acid formation and superoxide anion generation while its parent compound, salicylic acid (**3**), did not show this inhibitory activity [7]. It suggests that the presence of hydrophobic interaction between the alk(en)yl side chain of anacardic acid and xanthine oxidase was evidently associated with the activity. Similar observations were found between cardols, 5-pentadecatrienylresorcinol (**2**) and resorcinol (**4**). However, the rationale for this, especially the role of the hydrophobic portion, is still poorly understood. About the inhibitory activity of superoxide anion generation catalyzed by xanthine oxidase, it is known that the activity consisted of inhibition of xanthine oxidase activity, scavenging of superoxide anion generated and suppression caused by reduction of xanthine oxidase [8, 9].

Instead of anacardic acid and cardol derivatives, gallic acid (**5**) and its alkyl esters (**6**–**13**) are really suited for the study of the enzymic interaction because they can be easily prepared. In addition, propyl, octyl, and dodecyl gallates (**7**, **9**, and **11**) are currently permitted for use as antioxidant additives in food products. These prospectives prompted us to investigate the inhibitory effect of gallic acid (**5**) and its alkyl esters (**6**–**13**) on xanthine oxidase.

Correspondence: Professor Noriyoshi Masuoka, Department of Life Science, Okayama University of Science, 1–1 Ridai-cho Okayama, 700–0005, Japan
E-mail: masuokan@dls.ous.ac.jp
Fax: +81-86-256-9559

Abbreviations: FAD, flavin adenine dinucleotide; **NADH,** reduced form of nicotinamide adenine dinucleotide; **NBT,** nitroblue tetrazolium; **PMS,** phenazine methoxysulfate

2 Materials and methods

2.1 Chemicals

Gallic acid (**5**), *N*,*N*′-dicyclohexylcarbodiimide (DCC), a series of primary alcohols, menthol, borneol, xanthine,

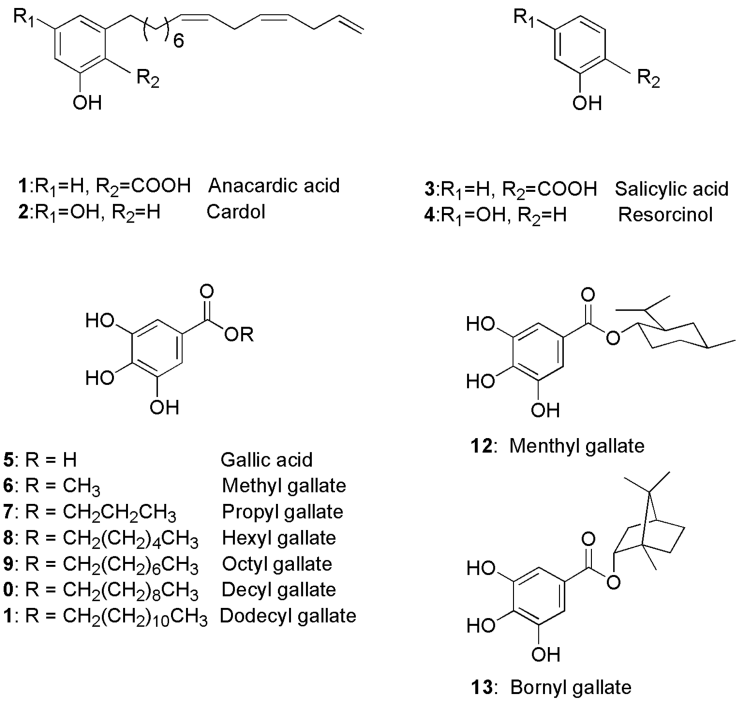

Figure 1. Chemical structures of gallic acid (**5**) and related compounds

nitroblue tetrazolium (NBT), phenazine methoxysulfate (PMS), and all solvents were purchased from Aldrich Chemical (Milwaukee, WI). BSA, reduced form of nicotinamide adenine dinucleotide (NADH) and other chemicals were obtained from Sigma Chemical (St. Louis, MO).

2.2 Synthesis

To a solution of 2.00 mM gallic acid and 2.00 mM alcohol in 6 mL of THF cooled at 0°C was added 4.2 mM DCC solution of in 6 mL of THF. After the solution had been allowed to stir for 20 h, the solvent was removed under reduced pressure. The residue was extracted with ethyl acetate several times and filtered. The filtrate was washed successively with dilute aqueous citric acid solution, saturated aqueous $NaHCO_3$ solution and water, dried over $MgSO_4$, and evaporated. The crude products were purified by chromatography (SiO_2; elution with $CHCl_3$–methanol, 98:2 v/v). Structures of the synthesized esters were established by spectroscopic methods (infrared, MS, and NMR). The best yield (87%) was obtained with octyl gallate (**9**) and the spectroscopic data of a series of alkyl gallates synthesized have recently been described [10]. Their analogs (**6**–**11**), menthyl gallate (**12**), and bornyl gallate (**13**), were also synthesized in the same manner. It should be noted that synthesis was achieved up to eicosanyl (C_{20}) gallate but the assay data were obtained unequivocally only up to dodecyl (C_{12}) gallate (**11**) because of solubility problems of the esters having more than 13 carbon atoms in the water-based test media.

Menthyl gallate (**12**) was obtained in 46% yield as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.76 (d, J = 7.0 Hz, 3H), 0.90 (d, J = 7.0 Hz, 3H), 0.92 (d, J = 7.0 Hz, 3H), 1.10 (m, 2H), 1.52 (m, 2H), 1.72 (m, 2H), 1.93 (m, 1H), 2.07 (m, 1H), 4.86 (td, J = 8.8, 3.6 Hz), 6.82 (bs, 3H), 7.32 (s, 2H). IR (nujol) 3345, 2930, 1670, 1545, 1450, 1325, 1030/cm. EI-MS (*m/z*) 309 ($M + H^+$).

Bornyl gallate (**13**) was obtained in 38% yield as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (s, 3H), 0.91 (s, 3H), 0.96 (s, 3H), 1.12 (m, 1H), 1.29 (m, 1H), 1.39 (m, 1H), 1.73 (m, 1H), 1.79 (m, 1H), 2.10 (m, 1H), 2.45 (m, 1H), 5.04 (dt, J = 10.0, 3.0 Hz), 6.21 (bs, 3H), 7.35 (s, 2H). IR (nujol): 3340, 2930, 1660, 1540, 1450, 1330, 1060/cm. EI-MS (*m/z*): 307 ($M + H^+$).

2.3 Sample solution

Gallic acid and alkyl gallates were dissolved with DMSO, and each 10 mM solution was prepared.

2.4 Xanthine oxidase reaction

The xanthine oxidase (EC 1.1.3.22, Grade IV) used for the bioassay was purchased from Sigma Chemical. The enzyme reaction was performed at pH 10 thoroughly in order to detect labile superoxide anions. Reaction mixture (final volume was 3.0 mL) containing 0–200 μM xanthine and 0.06 mL of sample solution in 40 mM sodium carbonate

buffer containing 0.1 mM EDTA (pH 10.0) was prepared under air atmosphere [7]. The reaction was started by adding 0.04 units of xanthine oxidase and completed at 25°C after 60 s. Reaction at various concentrations of oxygen was carried out at 200 μM xanthine in 3 mL of cuvette with a screw cap. Concentration of oxygen in the reaction mixture was adjusted by using nitrogen gas (passing through the mixture for 4 min) and addition of air-saturated buffer. Control experiments carried out by replacing the sample solution with the same amount of DMSO. Reaction rate was calculated from the proportional increase of the absorbance. Inhibition kinetics of the reaction was analyzed as a single substrate–a single inhibitor as the enzyme was saturated with another substrate.

2.4.1 Assay of uric acid

Assay of uric acid was performed by recording the absorbance at 293 nm of uric acid.

2.4.2 Assay of superoxide anion

Assay of superoxide anion under atmosphere was performed by addition of 25 μM NBT and 50 μg of BSA to the reaction mixture, and the absorbance at 560 nm (of formazan) was recorded.

2.4.3 Assay of oxygen consumption

The reaction was carried out at 200 μM xanthine in the reaction vessel of YSI 5300 (Yellow Springs Instruments, USA). Oxygen consumption was recorded with an OBH 100 oxygen electrode for 2 min, and the rate was calculated from the linear decrease.

2.4.4 Assay of hydrogen peroxide

The enzyme reaction was carried out at 200 μM xanthine in the presence of 20 μM gallic acid. After 0, 30, and 60 s, 0.2 mL of the reaction mixture was taken out and was immediately put into the test tube containing 0.2 mL of a reagent solution (64.5 μM *meso*-tetrakis(4-methylpyridyl)-porphinatoiron(III) pentachloride, 13.3 mM *N*,*N*-dimethylaniline, 2.76 mM 3-methyl-2-benzothiazolinone hydrazone, and 1.0 mM EDTA in 0.13 M hydrochloric acid) for stopping the reaction and determination of hydrogen peroxide [11]. The mixture was incubated at 25°C for 1 h, and then the absorbance at 590 nm was recorded.

2.5 Radical scavenging activity on superoxide anion generated by PMS-NADH system

Superoxide anion was generated nonenzymatically by a PMS-NADH system [12]. The reaction mixture (final volume was 3.0 mL) containing 25 μM NBT, 50 μg of BSA, 78 μM NADH, and 0.06 mL of sample solution in 40 mM sodium carbonate buffer containing 0.1 mM EDTA (pH 10.0) was prepared and incubated at 25°C for 3 min. Then, 0.03 mL of 15.5 μM PMS was added to the mixture, and the absorbance at 560 nm was recorded for 60 s (by formation of blue formazan). As control, 0.06 mL of DMSO was used. The reaction rate was calculated from the proportional increase of absorbance, and scavenging activity was expressed as percentage of the rate scavenged by gallates (rate of the control is 100%).

2.6 Molecular modeling

All molecular modeling was performed with a Chem 3D Pro Ver 4.5 obtained from Cambridge Soft (Cambridge, MA) for inhibitors of protein. The 3-D structure of xanthine oxidase was obtained from the PDB archive (http://www.rcsb/pdb/; PDB code: 1FIQ). The 3-D coordinates were obtained by visual docking of the inhibitors after the energy minimization process by MM2. In active site, the aromatic ring of gallate was overlapped with that of salicylic acid as an inhibitor involved in crystallized xanthine oxidase.

3 Results

3.1 Analysis of uric acid formation by xanthine oxidase reaction in the presence of gallic acid and alkyl gallates

Rates of uric acid formation at 200 μM xanthine were 158 ± 33 nM/s, and K_m for xanthine is 20.7 ± 3.4 μM. Subsequently, the effects of gallic acid (**5**) and alkyl gallates (**6**–**11**) on uric acid formation were examined, and the inhibition kinetics parameters are listed in Table 1. Among the alkyl gallates tested, dodecyl gallate (**11**) showed the most potent inhibitory activity for this oxygen-atom-transfer reaction with an IC_{50} of 49 ± 13 μM, followed by decyl gallate (**10**) with an IC_{50} of 97 ± 3 μM. The gallates with a longer alkyl chain showed more potent uric acid formation inhibitory activity than the shorter ones. The inhibition kinetics of uric acid formation with octyl gallate (**9**) was analyzed by Lineweaver–Burk plots as shown in Fig. 2. Octyl gallate was nearly a competitive inhibition ($K_I << K_{IS}$) for the xanthine-binding site, but decyl and dodecyl gallates were mixed-type inhibitors, with predominantly competitive inhibition ($K_I < K_{IS}$) for xanthine [13]. Gallates having a bulky group, menthyl gallate (**12**) and bornyl gallate (**13**), did not inhibit uric acid formation.

Uric acid formation catalyzed by xanthine oxidase was examined at various concentrations of oxygen. V_{max} value at 200 μM xanthine and K_m for oxygen were calculated as

Table 1. Kinetic parameters of gallic acid and its alkyl esters for uric acid formation by xanthine oxidase[a)]

Gallates tested	IC_{50} (μM)[b)]	For xanthine-binding (μM)[c)]		For oxygen-binding (μM)[d)]	
		K_I	K_{IS}	K_I	K_{IS}
Gallic acid (**5**)	>200	–	–	–	–
C_1 (**6**)	>200	–	–	–	–
C_3 (**7**)	>200	–	–	–	–
C_6 (**8**)	>200[e)]	–	–	–	–
C_8 (**9**)	262 ± 45[f)]	30.5 ± 1.8	943 ± 272	54.7 ± 0.6	902 ± 73
C_{10} (**10**)	97 ± 3	35.8 ± 11.8	127 ± 23	45.7 ± 12.5	128 ± 35
C_{12} (**11**)	49 ± 13[f)]	19.0 ± 6.0	46.7 ± 14.0	26.8 ± 3.1	111 ± 24
(**12**)	>200	–	–	–	–
(**13**)	>200	–	–	–	–

a) Values were expressed as mean ± SD obtained from three separate experiments.
b) IC_{50} values were indicated at 200 μM xanthine.
c) Xanthine oxidase reaction was carried out at 0–200 μM xanthine in the presence of gallates under atmosphere.
d) Xanthine oxidase reaction at 200 μM xanthine was carried out at 37.5–250 μM oxygen in the presence of gallates.
e) Hexyl gallate at 200 μM inhibited 13 ± 2% of the activity.
f) The value was estimated.

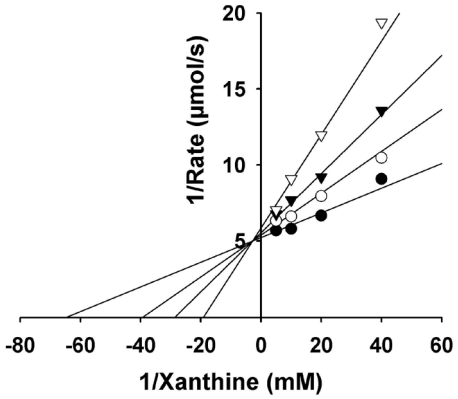

Figure 2. Lineweaver–Burk plots of uric acid formation by xanthine oxidase at 0–200 μM xanthine in the presence of octyl gallate. Concentrations of octyl gallate are 0 (●), 25 (○), 50 (▼), and 100 (▽) μM, respectively. Each symbol indicated is an average of three determinations.

178 ± 4 nM/s and 59.1 ± 8.4 μM, respectively. Results of the suppression with gallic acid and alkyl gallates were indicated in Table 1. Octyl gallate (**9**), decyl gallate (**10**), and dodecyl gallate (**11**) competitively inhibit uric acid formation for oxygen as these gallates inhibit that for xanthine. These results may be explained by the fact that octyl gallate nearly has two binding sites, xanthine- and oxygen-binding sites, in xanthine oxidase and that decyl and dodecyl gallates have one more binding site. As K_I values for xanthine is smaller than those for oxygen, alkyl gallates bind to the xanthine-binding site rather than to the oxygen-binding site. The oxygen-binding site of alkyl gallates may be close to the flavin adenine dinucleotide (FAD)-binding domain, which is considerably hydrophilic, and the structure of the site is similar to that of vanillyl alcohol oxidase, of which the isoalloxazine ring binds to a wide range of phenolic substrates [14], and galloyl esters bound to the isoalloxazine ring of FAD in squalene epoxidase [15]. As both K_{IS} values obtained from octyl, decyl, and dodecyl gallates for xanthine and oxygen decreased when the alkyl chain length increased, interaction of another binding site on xanthine oxidase increases when alkyl gallate has a longer (C_8<) chain. It should be noted that the measurement of uric acid formation in the presence of gallic acid and alkyl gallates was only possible for concentrations less than 0.2 mM due to overlapping absorbance of these compounds and uric acid at 293 nm.

3.2 Analysis of superoxide anion generation by xanthine oxidase reaction in the presence of gallic acid and alkyl gallates

Rates of superoxide anion generation at 200 μM xanthine are 184 ± 27 nM/s, and K_m for xanthine was 23.7 ± 4.9 μM. The kinetics was examined in the presence of 0.0, 0.5, 1.0, and 2.0 μM gallic acid or alkyl gallates. As a result, gallic acid (**5**) and all alkyl gallates (**6**–**13**) suppressed superoxide anion generation and the kinetic parameters obtained were listed in Table 2. The result indicates that gallic acid and alkyl gallates act as stronger inhibitors for superoxide anion generation than those for uric acid formation.

Kinetics of superoxide anion generation at various concentrations of oxygen could not be investigated by using the present method since NBT was directly reduced with xanthine–xanthine oxidase system at extremely low oxygen concentration (data not shown).

Table 2. Kinetic parameters of gallic acid and its alkyl esters for superoxide anion generation by xanthine oxidase[a)]

Gallates tested	IC_{50} (μM)[b)]	For xanthine-binding (μM)	
		K_I	K_{IS}
Gallic acid (**5**)	2.6 ± 0.3	1.5 ± 0.2	2.9 ± 0.3
C_1 (**6**)	7.4 ± 0.5	5.7 ± 0.4	8.0 ± 0.6
C_3 (**7**)	6.4 ± 1.0[c)]	4.5 ± 0.5	6.8 ± 0.7
C_6 (**8**)	5.2 ± 0.2[c)]	3.8 ± 0.4	5.3 ± 0.6
C_8 (**9**)	4.5 ± 0.5[c)]	3.9 ± 0.2	4.7 ± 0.2
C_{10} (**10**)	3.9 ± 1.1[c)]	2.5 ± 0.6	4.8 ± 1.0
C_{12} (**11**)	3.6 ± 0.2[c)]	1.8 ± 0.2	4.4 ± 0.2
(**12**)	4.9 ± 1.3[c)]	2.6 ± 0.4	4.9 ± 0.8
(**13**)	6.4 ± 1.0[c)]	4.5 ± 0.3	6.3 ± 0.4

a) Xanthine oxidase reaction was carried out at 0–200 μM xanthine in the presence of 0.0, 0.5, 1.0, and 2.0 μM of gallic acid or alkyl gallates under air atmosphere. Values were expressed as mean ± SD obtained from more than three separate experiments.
b) IC_{50} values were indicated at 200 μM xanthine.
c) The value was estimated.

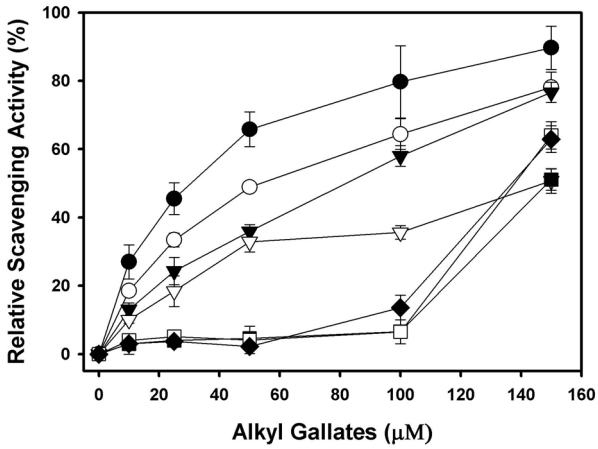

Figure 3. Scavenging of superoxide anion generated by PMS-NADH system with gallic acid and alkyl gallates. Scavenging activities indicated are gallic acid (●), methyl gallate (○), propyl gallate (▼), hexyl gallate (▽), octyl gallate (■), decyl gallate (□), and dodecyl gallate (♦). Vertical lines indicated the SD of more than three determinations.

3.2.1 Radical scavenging activity on superoxide anion generated by PMS-NADH system

To estimate the scavenging activity of superoxide anion by gallic acid and alkyl gallates, superoxide anion was generated nonenzymatically by using the PMS–NADH system (190 ± 13 nM/s). The result is indicated in Fig. 3. Among the compounds tested, gallic acid (**5**) was found to have the most effective scavenging activity with an IC_{50} of 29 ± 6 μM, followed by methyl gallate (**6**) with an IC_{50} of 51 ± 1μM. Two micromolar gallic acid scavenged 4.3 ± 0.4% of superoxide anion. Apparently, alkyl gallate having a shorter alkyl chain showed higher scavenging activity for superoxide anion. Alkyl gallates having a long alkyl chain ($\geq C_8$) revealed low scavenging activity but drastically increased the activity at more than 100 μM. Low scavenging activity of alkyl gallates having a longer chain may be explained with their low solubility in aqueous solution, and the increased scavenging activity at high concentration (>100 μM) may be associated to the formation of the soluble micelle [16]. The scavenging activity is low and different compared to their scavenging activity for superoxide anion generated by xanthine oxidase (Table 2). The difference is due to the fact that the latter contains not only scavenging activity of superoxide anion but also inhibition of xanthine oxidase activity and suppression of superoxide anion generation by the modification of xanthine oxidase.

3.2.2 Oxygen consumption at 200 μM xanthine

To confirm a xanthine oxidase reaction, consumption of oxygen by xanthine oxidase reaction was measured at 200 μM xanthine in the presence of 200 μM gallic acid or alkyl gallates. Decyl gallate (**10**), hexyl gallate (**8**), and gallic acid (**5**) suppressed 70 ± 2, 26 ± 6, and 2 ± 6% of oxygen consumption, respectively. Under the same conditions, decyl gallate, hexyl gallate, and gallic acid suppressed 74 ± 3, 27 ± 4, and 3 ± 1% of uric acid formation, respectively. The result indicates that oxygen consumption catalyzed by xanthine oxidase corresponds to the uric acid formation.

3.2.3 Hydrogen peroxide formation at 200 μM xanthine

To confirm a xanthine oxidase reaction, hydrogen peroxide generation rates in the presence of 0 and 20 μM gallic acid (**5**) were found to be 163 ± 4 and 180 ± 11 μM/s, respectively. Gallic acid did not suppress hydrogen peroxide formation by xanthine oxidase but slightly enhanced the generation. The result suggests that the maximum part of superoxide anion is spontaneously disproportionate and some part is reduced to hydrogen peroxide with gallic acid, which is oxidized to the corresponding *o*-quinone [17]. Under the same conditions, gallic acid suppressed 2.2 ± 4.0% of oxygen consumption, 1.0 ± 1.0% of uric acid formation and 83.2 ± 4.5% of superoxide anion generation. Accordingly, inhibition of superoxide anion generation does not correspond to that of xanthine oxidase reaction.

4 Discussion

In uric acid formation catalyzed by xanthine oxidase, the inhibitory effect was only observed by alkyl gallates having a longer chain (C_6) in all the gallic acid derivatives (Table

1). The results of the kinetics experiments indicate that these gallates preferably bind to the xanthine-binding site.

The reaction of uric acid formation by xanthine oxidase proceeds *via* transfer of an oxygen atom to xanthine from the molybdenum center. X-ray crystallographic analysis of bovine milk xanthine oxidase indicated the binding of bicyclic substrates, *e.g.*, with Phe 1009 perpendicular to the six-membered ring of xanthine and Phe914 stacking flat on top of the substrate's five-membered ring, which would then be able to form a covalent bond with one of the molybdenum ligands [18]. As octyl gallates having a C_8 chain is almost a competitive inhibitor for xanthine (Fig. 2) and other gallates are predominantly competitive inhibitors, these gallates can interact with Phe 1009 perpendicular to the pyrogallol moiety and Phe914 stacking flat on top of the alkyl group. The alkyl chain tightly comes in the hydrophobic protein pocket to act as an anchor and efficiently inhibits uric acid formation.

To confirm this postulation, the effect of menthyl gallate (**12**) and bornyl gallate (**13**) on xanthine oxidase activity was examined. As expected, both compounds insignificantly inhibited uric acid formation. Since the bulky cyclic monoterpene moieties, which are exposed on the other side of the molecules, cannot be well embraced by the hydrophobic protein pocket near the xanthine-binding site. Furthermore, as illustrated in Fig. 4, the docking experiment of octyl gallate to xanthine oxidase using the X-ray crystallographic result also supported the possibility to fit the long alkyl chain of gallates to the xanthine-binding site rather than the short chain and the bulk moiety [18].

On the other hand, all alkyl gallates equally suppress superoxide anion generation by xanthine oxidase at low concentrations. As it has been reported that these alkyl gallates directly scavenge radical species such as 1,1-diphenyl-2-picrylhydrazyl (DPPH radical) [17], the scavenging activity of superoxide anion nonenzymatically generated by PMS-NADH system was examined. Gallic acid showed the most potent scavenging activity but the scavenging activity is low at 2 μM (Fig. 3). As kinetics experiments of superoxide anion generation by xanthine oxidase are carried out at limited concentrations (0–2 μM) of gallic acid and its esters, the results (Table 2) indicate that their inhibitory activity is not mainly due to their scavenging activity and is independent of alkyl chain lengths and bulky groups of these gallates. This suggests that their activity depends upon galloyl moiety in these gallates.

As gallic acid did not inhibit uric acid formation but superoxide anion generation by xanthine oxidase, oxygen consumption and hydrogen peroxide formation of the reaction were determined in the presence of gallic acid. The result confirms that the inhibition of superoxide anion generation does not correspond to that of xanthine oxidase reaction and is due to the formation of hydrogen peroxide.

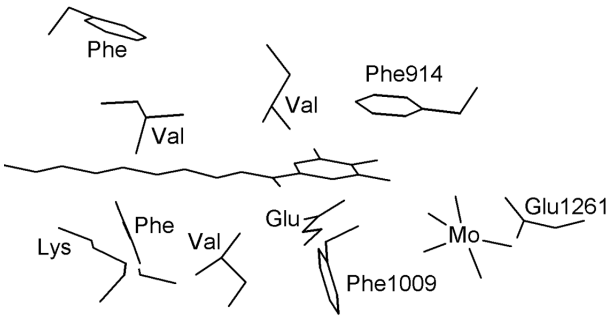

Figure 4. Docking of octyl gallate to xanthine-binding site in xanthine oxidase. Octyl gallate can access to Phe 1009 perpendicular to the pyrogallol moiety and Phe914 stacking flat on top of the octyl group. Amino acid residues around the alkyl chain of octyl gallate indicate the hydrophobic protein pocket, and the alkyl chain interacts with them.

From these results, we deduced that the inhibition is due to modification of the enzyme by the gallate derivatives. It is known that xanthine oxidase takes several oxidation states, and fully reduced xanthine oxidase contains a total of six electrons in the reduced iron–sulfur center, molybdenum (IV), and $FADH_2$. Oxidation of four or six electron-reduced xanthine oxidase to two electron-reduced xanthine oxidase generates only hydrogen peroxide, and that of two electron-reduced xanthine oxidase generates superoxide anion and hydrogen peroxide [8, 9]. Therefore, it is deduced that alkyl gallates reduced xanthine oxidase to four or six electron-reduced xanthine oxidase when these gallates are added to a mixture of xanthine oxidase reaction. The reduced enzyme undergoes a heterolytic rather than homolytic cleavage of the flavin hydroperoxide, a flavin-oxygen intermediate of the xanthine oxidase reaction, to produce hydrogen peroxide [9]. Compared to these gallates, anacardic acid (**1**) cannot suppress superoxide anion generation by xanthine oxidase at low concentration since the acid is not a reducing agent [7]. Further study is progressing about cardols (**2**).

This work was supported in part by Grant-in-Aid 15651098 for NM from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

5 References

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