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Inhibitory Effects of Green Tea Catechins on the Activity of Human Matrix Metalloproteinase 7 (Matrilysin)

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Inhibitory effects of green tea catechins and their derivatives on the matrilysin-catalyzed hydrolysis of a synthetic substrate, (7-methoxycoumarin-4-yl)acetyl-L-Pro-L-Leu-Gly-L-Leu-[N^3-(2,4-dinitrophenyl)-L-2,3-diamino-propionyl]-L-Ala-L-Arg-NH_2 [MOCAc-PLGL(Dpa)AR], were examined. The 10 catechins examined were classified into three groups according to their inhibition potency. Catechins with a galloyl group at the 3 position, including a major component of green tea catechin, (–)-epigallo-3-catechin gallate [(–)-EGCG], were the most potent inhibitors and inhibited matrilysin in a non-competitive manner with K_i values of 0.47–1.65 μM. The inhibitory potency of (–)-EGCG was not influenced by the presence of an inhibitor, $ZnCl_2$, suggesting that the inhibitions of matrilysin by (–)-EGCG and by $ZnCl_2$ might be independent of each other. The inhibitory effects of green tea catechins suggest that a high intake of green tea might be effective for the prevention of tumor metastasis and invasion in which matrilysin is concerned.

Key words: catechin, inhibitor, matrilysin, matrix metalloproteinase.

Abbreviations: BHT, butylated hydroxytoluene; (+)-C, (+)-catechin; (–)-C, (–)-catechin; (–)-CG, (–)-catechin gallate; (+)-EC, (+)-epicatechin; (–)-EC, (–)-epicatechin; (–)-EGC, (–)-epigallocatechin; (–)-ECG, (–)-epicatechin-3-gallate; (–)-EGCG, (–)-epigallocatechin-3-gallate; (–)-GC, (–)-gallocatechin; (–)-GCG, (–)-gallocatechin-3-gallate; IC_{50}, the inhibitor concentration required to inhibit the enzyme activity to 50% of that observed in the absence of the inhibitor; MOCAc-PLG, (7-methoxycoumarin-4-yl) acetyl-L-Pro-L-Leu-Gly; MOCAc-PLGL(Dpa)AR, MOCAc-L-Pro-L-Leu-Gly-L-Leu-[N^3-(2,4-dinitrophenyl)-L-2,3-diamino-propionyl]-L-Ala-L-Arg-NH_2.

Matrix metalloproteinases (MMPs) comprise a family of zinc endopeptidases that are believed to degrade extracellular matrix (ECM) and take part in both normal and pathological processes, including development, reproduction, maintenance, tissue destruction, fibrotic diseases, and weakening of the matrix (*1*, *2*).

Matrilysin (MMP-7)[EC 3.4.24.23] is the smallest MMP, consisting of a signal peptide, a propeptide, and a catalytic domain; the molecular mass of the latent proform is 28 kDa and that of the mature form is 19 kDa (*3*, *4*). X-ray crystallographic analyses of recombinant human matrilysin in complexes with inhibitors have demonstrated that matrilysin is composed of a five-stranded β-sheet and three α-helices, and contains a zinc ion essential for enzyme activity, as well as another zinc ion and two calcium ions that are regarded as necessary for enzyme stability (*5*). Matrilysin has been detected in lesions of the prostate (*6*), colon (*7*), brain (*8*), stomach (*9*), lung (*10*), and breast (*11*), and degrades ECM components including gelatins of type I, III, IV, and V, type IV basement membrane collagen, fibronectin, vitronectin, proteoglycan, laminin, and elastin (*4*, *12–14*), suggesting that matrilysin may play a role in tumor invasion and metastasis. From this viewpoint, the development of matrilysin inhibitors is considered to be of therapeutic benefit.

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Preparing large quantities of human matrilysin is indispensable for studying its structure-function relationships and for the development of inhibitors that could be useful for cancer therapy. We have previously proposed an effective procedure to prepare active matrilysin from inclusion bodies expressed by *Escherichia coli* in good yield and at reasonable cost (*15*). We have studied the stability and denaturation of matrilysin, the state of tryptophyl residues (*16*), the effects of dimethyl sulfoxide (DMSO), temperature, and sodium chloride on matrilysin activity (*17*), and the interaction of matrilysin with the synthetic inhibitors thiorphan and R-94138 (*18*). It has been suggested that hydrophobic interactions at the active site of matrilysin are significant for the recognition by matrilysin of substrate and inhibitors (*17*, *18*).

Recently, various physiological activities, such as hepatotoxic, anti-nutritional, carcinogenic, anti-mutagenic, anti-microbial, anti-viral, and immunomodulating activities, of green tea catechins have been noted (*19*). It has been reported that a major component of green tea catechin, (–)-epigallocatechin-3-gallate [(–)-EGCG], induces apoptosis in cancer cells (*20–22*), inhibits angiogenesis and metastasis as a direct inhibitor of MMPs (*23–27*), and down-regulates the expression of MMP-2 and MMP-9 (*25*). These observations strongly suggest that green tea catechins may be effective in cancer prevention.

In the present paper, we demonstrate the inhibitory effects of various green tea catechins on matrilysin activity.

MATERIALS AND METHODS

Materials—Human matrilysin was prepared according to the methods previously reported (*15*, *17*). The concentration of matrilysin was determined with a Shimadzu UV-2200 spectrophotometer (Kyoto) using the molar absorption coefficient at 280 nm of 31.8 $mM^{-1}\cdot cm^{-1}$ calculated from the amino acid composition (*3*). A substrate of matrilysin, (7-methoxycoumarin-4-yl)acetyl-L-Pro-L-Leu-Gly-L-Leu-[N^3-(2,4-dinitrophenyl)-L-2,3-diamino-propionyl]-L-Ala-L-Arg-NH_2 [MOCAc-PLGL(Dpa)AR], is known to be cleaved at the peptide bond between the glycine and leucine residues (*17*, *28*). MOCAc-PLGL(Dpa)AR (Lot 480429) and (7-methoxycoumarin-4-yl)acetyl-L-Pro-L-Leu-Gly [MOCAc-PLG] (Lot 471218) were purchased from the Peptide Institute (Osaka), and their concentrations were determined using the molar absorption coefficients ε_{410} = 7.5 $mM^{-1}\cdot cm^{-1}$ and ε_{324}=12.9 $mM^{-1}\cdot cm^{-1}$, respectively (*28*). (+)-Catechin [(+)-C] (Lot 58H1174), (–)-catechin [(–)-C] (Lot 29H1263), (–)-gallocatechin [(–)-GC] (Lot 88H0837), (–)-epigallocatechin [(–)-EGC] (Lot 79H0923), and tannic acid (Lot 59H0342) were from Sigma (St. Louis, MO). (+)-Epicatechin [(+)-EC] (Lot 98J28), (–)-epicatechin [(–)-EC] (Lot 13731JR), (–)-catechin-3-gallate [(–)-CG] (Lot C01120101E08), (–)-epicatechin-3-gallate [(–)-ECG] (Lot C001201C09), (–)-gallocatechin-3-gallate [(–)-GCG] (Lot C011401I10), (–)-epigallocatechin-3-gallate [(–)-EGCG] (Lot C001401I10), and polyphenon 100 (Lot KU-0001-00) were from Funakoshi (Tokyo). Polyphenon 100 is a mixture of various catechins with a total catechin content of 81.3% (w/w); the catechin components comprised (–)-EGCG (53.9%), (–)-EGC (13.4%), (–)-EC (9.4%), (–)-GCG (2.9%), and (–)-ECG (1.7%) according to the data of the supplier. Methyl gallate (Lot 07909MS) was from Aldrich (Milwaukee, WI). All other chemicals were of reagent grade and purchased from Nacalai Tesque (Kyoto). The HPLC apparatus, consisting of a solvent delivery system CCPM-II, a UV monitoring system UV-8020, a computer control system PX-8020, a degasser SD-8020, a column oven CO-8020, and an integrator Chromatocoder 21, was purchased from Tosoh (Tokyo).

Fluorometric Analysis of the Matrilysin-Catalyzed Hydrolysis of MOCAc-PLGL(Dpa)AR—After mixing 3696 μl of matrilysin (10.1 nM) dissolved in 50.7 mM HEPES buffer (pH 7.5) plus 10.1 mM $CaCl_2$ and 30 μl of catechin (0–5.65 mM) dissolved in ethanol plus 0.1% BHT, the reaction mixture was incubated at pH 7.5, 25°C, for 30 min. The hydrolysis of MOCAc-PLGL(Dpa)AR was initiated by adding 8 μl of MOCAc-PLGL(Dpa)AR (234 μM) dissolved in DMSO to 1242 μl of the mixture, and measured by following the increase in the fluorescence intensity at 393 nm with excitation at 328 nm with a JASCO FP-777 fluorescence spectrophotometer (Tokyo) equipped with a cell-holder that maintains a constant temperature using a Peltier-effect element.

HPLC Analysis of the Matrilysin-Catalyzed Hydrolysis of MOCAc-PLGL(Dpa)AR—After mixing 942 μl of matrilysin (13.2 nM) dissolved in 53.1 mM HEPES buffer (pH 7.5) plus 10.6 mM $CaCl_2$ and 8 μl of catechin (0–647 μM) dissolved in ethanol plus 0.1% BHT, the reaction mixture was incubated at pH 7.5, 25°C, for 30 min. The hydrolysis of MOCAc-PLGL(Dpa)AR was initiated by adding 50 μl of substrate (0.4–2.4 mM) dissolved in DMSO to the mixture. The initial concentration of matrilysin was 12.7 nM. The reaction was stopped by mixing 100 μl of the reaction solution with 400 μl of 1% trifluoroacetic acid (TFA). The mixture (100 μl) was applied to HPLC on a TSKgel ODS-80Ts column [4.6 mm (inner diameter) × 150 mm] (Tosoh, Tokyo) equilibrated with 0.1% TFA. A linear gradient was generated from 20 to 70% acetonitrile at time over 15 min starting at 5 min at a flow-rate of 1 ml/min, and the absorption of the eluate was monitored at 335 nm. The amount of product, MOCAc-PLG, was determined from the peak area. The initial reaction rate, v, at a substrate concentration was determined from the time course of the amount of MOCAc-PLG formed, and the kinetic parameters, the catalytic (k_{cat}) and Michaelis (K_m) constants, were determined according to the Michaelis-Menten equation using the nonlinear least-squares method (*29*).

Effect of $ZnCl_2$ on the Inhibition of Matrilysin by (–)-EGCG—After mixing 3568 μl of matrilysin (11.3 nM) dissolved in 56.4 mM HEPES buffer (pH 7.5) plus 11.3 mM $CaCl_2$ and 32 μl of (–)-EGCG (0–1.16 mM) dissolved in ethanol plus 0.1% BHT, the reaction mixture was incubated at pH 7.5, 25°C, for 30 min. After the addition of 400 μl of $ZnCl_2$ dissolved in water (pH 7.0) to the reaction mixture, the solution containing $ZnCl_2$ (0 or 100 μM) was further incubated at pH 7.5, 25°C, for 10 min. The hydrolysis of MOCAc-PLGL(Dpa)AR was initiated by adding 8 μl of substrate dissolved in DMSO to 1242 μl of the mixture, and measured by following the increase in the fluorescence intensity.

RESULTS

Inhibitory Effect of a Green Tea Extract, Polyphenon 100, on the Matrilysin-Catalyzed Hydrolysis of MOCAc-PLGL(Dpa)AR—Generally, the hydrolysis of MOCAc-PLGL(Dpa)AR is measured by following the increase in the fluorescence intensity at 393 nm with excitation at 328 nm, and the rate of the increase is converted into the reaction rate as measured for product formation using the fluorescence intensity of a known amount of the product, MOCAc-PLG. However, in the presence of a compound that absorbs the excitation light, the inner filter effect should be taken into consideration. In order to evaluate matrilysin activity precisely, the dependence of the fluorescence intensity of MOCAc-PLG on its concentration was examined in the presence of polyphenon 100 (Fig. 1). Although the fluorescence intensity (FI) increased linearly in proportion to the MOCAc-PLG concentration at three different concentrations of polyphenon 100, the intercept on the vertical axis increased, whereas the slope, defined as an increment of the fluorescence intensity against an increment of the MOCAc-PLG concentration ($\Delta FI/\Delta[MOCAc\text{-}PLG]_0$), decreased with increasing polyphenon 100 concentration. These plots suggest that polyphenon 100 absorbs the excitation light and emits a slight fluorescence. Thus, the fluorescence intensity of MOCAc-PLG is attenuated by the inner filter effect. The linearity of the plots was maintained at high concentrations of polyphenon 100 (15–45 μg/ml), indicating that MOCAc-PLG could be quantified even under these conditions. Accordingly, the matrilysin activity in the presence of catechin was corrected using the ΔFI/

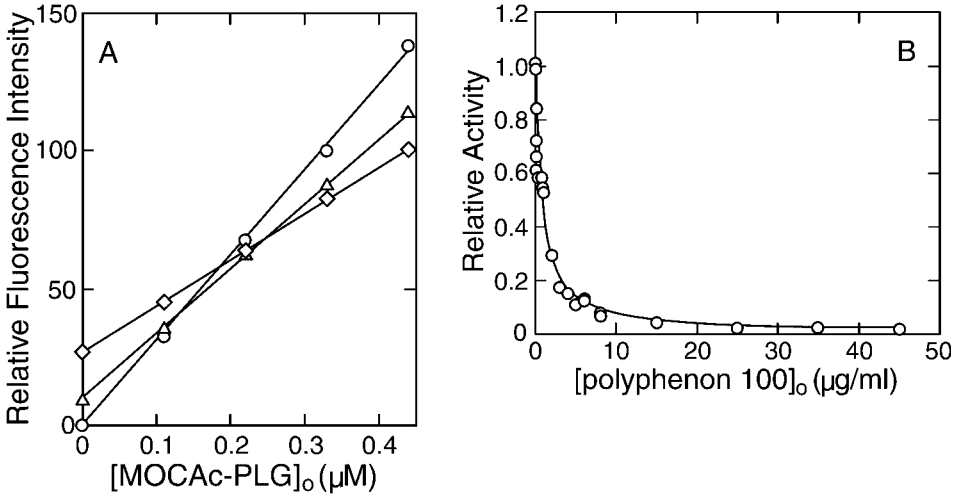

Fig. 1. **Evaluation of the inhibitory effect of polyphenon 100 on matrilysin activity.** Panel A: Effect of polyphenon 100 on the fluorescence intensity of MOCAc-PLG. The fluorescence intensity was measured with excitation at 328 nm and emission at 393 nm in 50 mM HEPES (pH 7.5) plus 10 mM $CaCl_2$, 0.8% EtOH, 8 × 10^{-4}% BHT, and 0.6% DMSO at 25°C. The initial concentrations of polyphenon 100 were 0 (open circles), 15 (open triangles), and 45 μg/ml (open diamonds). Panel B: Inhibitory effect of polyphenon 100 on matrilysin activity. The reaction was performed in the same buffer at 25°C. The initial concentrations of matrilysin and MOCAc-PLGL(Dpa)AR were 10.0 nM and 1.5 μM, respectively. The activity (v_0 = 1.98 nM·s^{-1}) obtained in the absence of polyphenon 100 was taken as 1.0. The IC_{50} value of polyphenon 100 was determined to be 0.84 ± 0.09 μg/ml.

Δ[MOCAc-PLG]$_o$ value. Polyphenon 100 strongly inhibited the matrilysin activity, and the inhibitor concentration (IC_{50}) at which 50% of the enzyme activity observed in the absence of inhibitor at pH 7.5, 25°C was determined to be 0.84 ± 0.09 μg/ml (Fig. 1).

Inhibitory Effect of (–)-EGCG on Matrilysin Activity— In order to investigate the inhibition mechanism of the major component of polyphenon 100, (–)-EGCG, the hydrolysis was monitored by HPLC rather than fluorometrically because of the absorptive quenching effect due to the dinitrophenyl group at high concentrations of MOCAc-PLGL(Dpa)AR. Five percent DMSO was added to the reaction solution in order to dissolve the substrate enough to determine k_{cat} and K_m separately (*17*). When $[S]_0/v$ was plotted against $[S]_0$ (Hanes-Woolf plot) at two different inhibitor concentrations, two lines crossed apparently at a point on the $[S]_0$-axis, suggesting that the inhibition of (–)-EGCG is non-competitive (Fig. 2). Based on this, the reaction rate (v) can be described as:

$$v = \frac{k_{cat}[E][S]}{(K_m + [S])\left(1 + \frac{[I]}{K_i}\right)} \quad (1)$$

where [I] is the inhibitor concentration and K_i is the inhibitor constant. Relative activity (v/v_0) is expressed as:

$$\frac{v}{v_0} = \frac{1}{1 + \frac{[I]}{K_i}} \quad (2)$$

where v_0 is the matrilysin activity in the absence of (–)-EGCG. As is clear from Eq. 2, it is reasonable to set the K_i value equal to the IC_{50} value under these conditions, and the inhibitor constant (K_i) of (–)-EGCG was calculated to be 1.14 μM from the slope of the plots. The K_m value under the conditions examined was also determined to be 62 μM from the intercepts of the two lines in the $[S]_0$-axis. The inhibitory effect was further examined fluorometrically by changing the inhibitor concentration up to 46 μM, and the K_i value was determined more precisely to be 1.65±0.03 μM from Eq. 2 using non-linear least-squares

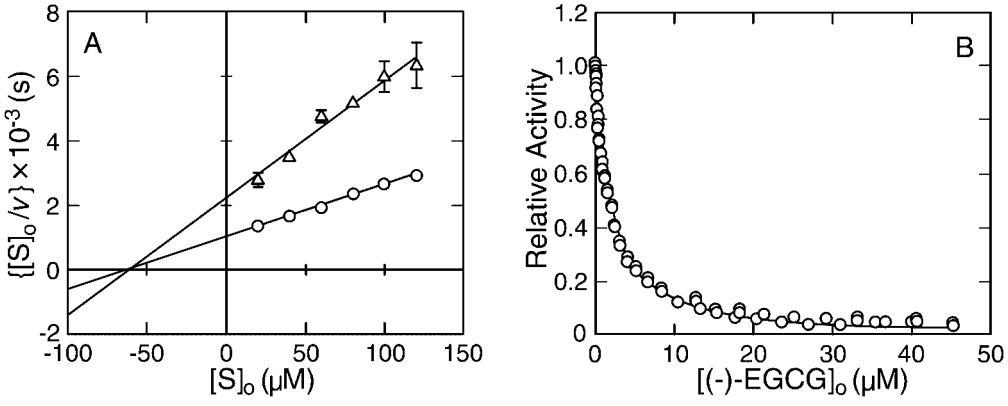

Fig. 2. **Inhibitory effect of (–)-EGCG on matrilysin activity.** Panel A: Hanes-Woolf plot of the inhibition of matrilysin activity by (–)-EGCG. The reaction was performed in 50 mM HEPES (pH 7.5) plus 10 mM $CaCl_2$, 0.8% EtOH, 8 × 10^{-4}% BHT, and 5% DMSO at 25°C. The initial concentration of matrilysin was 12.7 nM. The initial concentrations of (–)-EGCG were 0 (open circles) and 1.44 μM (open triangle). Panel B: Dependence of matrilysin activity on (–)-EGCG concentration. The reaction was performed in 50 mM HEPES (pH 7.5) plus 10 mM $CaCl_2$, 0.8% EtOH, 8 × 10^{-4}% BHT, and 0.6% DMSO at 25°C. The initial concentrations of matrilysin and MOCAc-PLGL(Dpa)AR were 10.0 nM and 1.5 μM, respectively. The activity (v_0 = 1.98 nM·s^{-1}) obtained in the absence of (–)-EGCG was taken as 1.0. The inhibitor constant (K_i) was determined to be 1.65 ± 0.03 μM. The solid line is a theoretical curve drawn according to Eq. 2 using a K_i value of 1.65 μM.

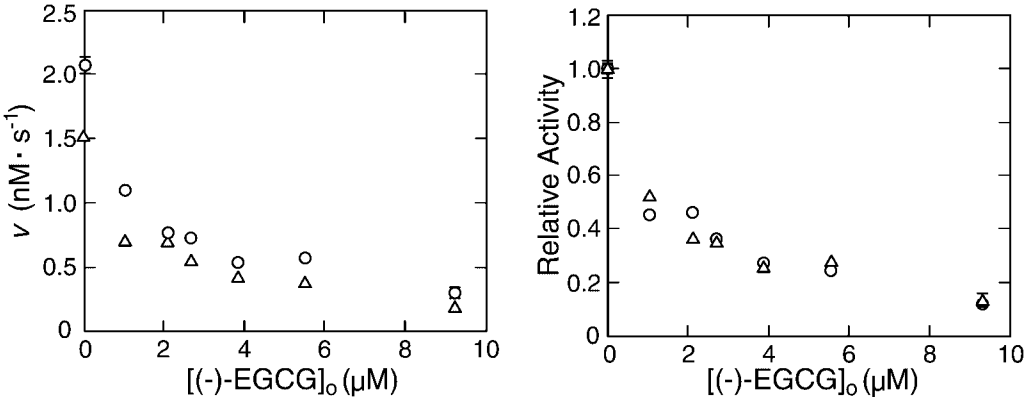

Fig. 3. **Effect of $ZnCl_2$ on matrilysin activity inhibited by (–)-EGCG.** The reaction was performed in 50 mM HEPES (pH 7.5) plus 10 mM $CaCl_2$, 0.8% EtOH, 8×10^{-4}% BHT, and 0.6% DMSO at 25°C. The initial concentrations of matrilysin and MOCAc-PLGL (Dpa)AR were 10.0 nM and 1.5 μM, respectively. The initial concentrations of $ZnCl_2$ were 0 (open circles) and 100 μM (open triangles). Panel A: Dependence of the enzyme activity, v, on (–)-EGCG concentration. Panel B: Dependence of the relative activity on (–)-EGCG concentration. The activities of 2.09 nM·s^{-1} in the absence of $ZnCl_2$ and 1.51 nM·s^{-1} in the presence of $ZnCl_2$ were taken as relative activities of 1.0.

regression (Fig. 2). This value is in good agreement with that obtained by HPLC analysis.

Effect of $ZnCl_2$ on the Inhibition of Matrilysin by (–)-EGCG—The inhibitory effect of (–)-EGCG on matrilysin activity was also examined in the presence of $ZnCl_2$. The enzyme activity observed in the presence of (–)-EGCG over the range of 0–9.5 μM was slightly (10–30%) decreased when 100 μM $ZnCl_2$ was added to the reaction mixture (Fig. 3). In the absence of (–)-EGCG, the activity without 100 μM $ZnCl_2$ was 2.09 nM/s and that with 100 μM $ZnCl_2$ was 1.51 nM/s, suggesting that 100 μM $ZnCl_2$ inhibits matrilysin activity by 28% in the absence of 100 μM $ZnCl_2$. The dependence of the relative activity on the (–)-EGCG concentration observed in the presence of 100 μM $ZnCl_2$ was almost the same as that observed in the absence of 100 μM $ZnCl_2$ instead of the inhibitory effect of $ZnCl_2$ (Fig. 3). This indicates that an excess amount of $ZnCl_2$ inhibits matrilysin, but that $ZnCl_2$ does not have a significant influence on the inhibitory activity of (–)-EGCG, and the degree of inhibition observed in the presence of both (–)-EGCG and $ZnCl_2$ is the sum of the degrees of inhibition caused by the respective inhibitors. Consequently, the inhibitions of matrilysin by (–)-EGCG and by $ZnCl_2$ might be independent of each other.

Inhibitory Effects of Catechins on Matrilysin Activity—The inhibitory effects of individual green tea catechins and their derivatives on matrilysin activity were also examined (Fig. 4, Table 1). According to their inhibition potency, the 10 catechins examined were classified into three groups. (+)-C, (–)-C, (+)-EC, and (–)-EC inhibited matrilysin activity with IC_{50} values greater than 1 mM. (–)-GC and (–)-EGC, catechins with a hydroxyl group at the 5′ position, showed IC_{50} values greater than 50 μM. Catechins with a galloyl group strongly inhibited matrilysin activity with IC_{50} (K_i) values of (–)-CG, (–)-ECG, and (–)-GCG determined to be 1.44 ± 0.07, 0.47 ± 0.04, and 1.06 ± 0.05 μM, respectively.

DISCUSSION

This study demonstrates that green tea catechins inhibit not only MMP-2 and MMP-9, but also matrilysin (MMP-7). Interestingly, the inhibition potency depends on the existence of a hydroxyl group at the 5′ position and a gal-

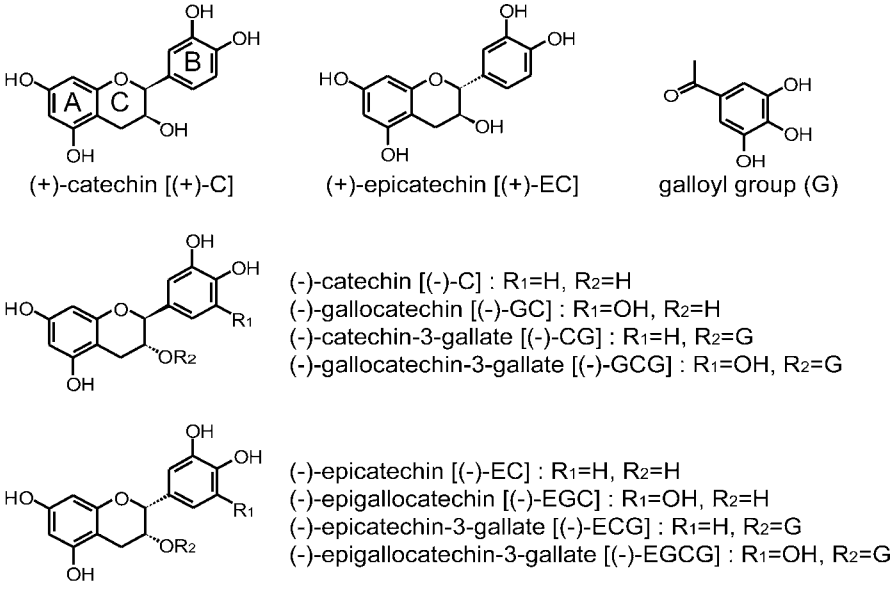

Fig. 4. **Molecular structures of the catechins examined.**

Table 1. **Inhibitory effects of catechins on matrilysin activity.**

Catechins	IC_{50} (K_i) (μM)
(+)-catechin [(+)-C]	>1000
(–)-catechin [(–)-C]	>1000
(+)-epicatechin [(+)-EC]	>1000
(–)-epicatechin [(–)-EC]	>1000
(–)-gallocatechin [(–)-GC]	>50
(–)-epigallocatechin [(–)-EGC]	>50
(–)-catechin-3-gallate [(–)-CG]	1.44 ± 0.07
(–)-epicatechin-3-gallate [(–)-ECG]	0.47 ± 0.04
(–)-gallocatechin-3-gallate [(–)-GCG]	1.06 ± 0.05
(–)-epigallocatechin-3-gallate [(–)-EGCG]	1.65 ± 0.03

The reactions were performed in 50 mM HEPES buffer (pH 7.5) plus 10 mM $CaCl_2$, 0.8% ethanol, 8×10^{-4}% BHT, and 0.6% dimethyl sulfoxide (DMSO) at 25°C. The initial concentrations of matrilysin and MOCAc-PLGL(Dpa)AR were 10.0 nM and 1.5 μM, respectively.

loyl group at the 3 position of the flavanol skeleton. The affinities of (–)-C and (–)-EC with matrilysin increase 20-fold when a hydroxyl group is introduced into the 5′ position [as shown with (–)-GC and (–)-EGC], and increase by 1,000-fold when a galloyl group is introduced into the 3 position [as shown with (–)-CG and (–)-ECG]. However, no synergistic effect of the hydroxyl and galloyl groups was observed. The inhibitory effects of four catechins containing a galloyl group on matrilysin activity are almost the same, suggesting that the stereo-isomerism among the four catechins has no effect on inhibition. On the other hand, no inhibition was observed by compounds with a galloyl group such as methyl gallate, tannic acid, or gallic acid, even at 100 μM (data not shown), suggesting that both the flavanol skeleton and the galloyl group are essential for the inhibition of matrilysin activity.

The inhibitory potency of (–)-EGCG against matrilysin activity was not significantly influenced by adding an excess amount (100 μM) of $ZnCl_2$, and the same level of the inhibition was observed for $ZnCl_2$ in the absence and presence of (–)-EGCG. The degree of inhibition observed in the presence of both inhibitors is the sum of the degrees of inhibition of the respective inhibitors. This suggests that the inhibition is not the result of the metal-chelating ability of (–)-EGCG against the catalytically essential zinc atom, and could be brought about by a direct interaction between the enzyme and (–)-EGCG. On the other hand, the inhibitions by (–)-EGCG and by $ZnCl_2$ are independent, suggesting that the binding sites for Zn^{2+} and (–)-EGCG might be separate. At present, we have no data concerning the Zn^{2+} binding site on the matrilysin molecule, although kinetic and spectrophotometric studies are now under way. It should be noted that a bacterial metalloproteinase, thermolysin, is also inhibited by excess amounts of Zn^{2+} ion, and the manner of inhibition was determined to be competitive, suggesting that Zn^{2+} may bind at the active site to inhibit enzyme activity [T. Watanabe: Master's Thesis, Kyoto University (2003)]. Considering this unpublished data and the analogous features of metalloproteinases, it is possible that Zn^{2+} may bind to matrilysin at the active site.

Both HPLC and fluorometric analyses indicate clearly that the manner of inhibition by (–)-EGCG is non-competitive, and the inhibition potency is independent of the presence of 0.6% or 5% DMSO. We previously reported that DMSO inhibits matrilysin activity in a competitive manner with a K_i value of 4.64% or 0.59 M (*17*). The competitive inhibition by DMSO and non-competitive inhibition by (–)-EGCG are considered to be independent. It has been reported that (–)-EGCG inhibits MMP-2 non-competitively with a K_i value of 22 μM (*27*). These observations suggest that (–)-EGCG binds to a region other than the active site of these MMPs regardless of the considerable difference in their affinities for (–)-EGCG. It has been reported that (–)-EGCG inhibits MMP-2 and MMP-9 with IC_{50} values of 6.0 and 0.3 μM, respectively, whereas (–)-ECG inhibits with IC_{50} values of 95 and 28 μM, respectively (*24*). A synergistic effect of the hydroxyl and galloyl groups is observed for the inhibition of MMP-2 and MMP-9, although such an effect is not observed for the inhibition of matrilysin. A study to examine in detail the interaction between the inhibitor and matrilysin and the inhibitor-binding site on the enzyme is currently underway using spectroscopic, calorimetric, and crystallographic analyses.

It has been reported that tea brews generally consumed contain 708 μg/ml (–)-EGCG, 324 μg/ml (–)EGC, 141 μg/ml (–)-ECG, 84 μg/ml (–)-EC, and 23 μg/ml (+)-C (*30*), and that the concentration of a major component, (–)-EGCG, in human plasma is 0.1–0.3 μM after drinking 2–3 cups of tea (*24*, *31*). This suggests that a high intake of green tea may be effective for the prevention of tumor metastasis and invasion by inhibiting matrilysin as well as MMP-2, MMP-3, and MMP-9. It has been reported that catechins and related polyphenols exhibit apoptosis-inducing activity in several cancer cell lines (*32*). Most of the MMP inhibitors so far developed are pseudopeptides with a catalytic zinc chelating group, and thus they inhibit MMPs competitively (*33*). Catechins are characteristic inhibitors in that they inhibit MMP non-competitively. These observations could be useful for the development of inhibitors based on flavanol for therapeutic use.

Our preliminary study has shown that dibenzylbutyrolactone lignans, especially matairesinol and its derivatives, inhibit matrilysin with IC_{50} values ranging from 50 to several hundred μM (submitted). The dibenzylbutyrolactone structure is essential for inhibition, and introducing methylene-dioxide rings and hydroxyl groups appears to enhance the inhibitory activity. Polyphenols are currently of great interest because of their antioxidative functions. The inhibitory effects of catechins as well as lignans on matrilysin remind us of the possibility that polyphenols might plausibly regulate biological reactions by inhibiting key-proteinases in addition to controlling oxidative conditions.

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