Note

DNA Cleavage Activities of (−)-Epigallocatechin, (−)-Epicatechin, (+)-Catechin, and (−)-Epigallocatechin Gallate with Various Kind of Metal Ions

Fumiko HAYAKAWA,1 Takahide KIMURA,2 Nobuo HOSHINO,3 and Takashi ANDO2

1Department of Life Style Studies, School of Human Cultures, The University of Shiga Prefecture, Hassaka-cho, Hikone, Shiga 522-8533, Japan
2Department of Chemistry and 3Department of Pharmacy, Shiga University of Medical Science, Seta, Otsu, Shiga 520-2192, Japan

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The DNA cleavage activities of (+)-catechin (C), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (EGCg) were examined with 16 different metal ions. Cu2+ with all the catechins facilitated DNA cleavage, while Ag+ with EGC and EC showed a strong repressive effect. The other metal ions examined showed little effect.

Key words: catechin; metal ion; DNA cleavage; antioxidant; prooxidant

The effects on the repression of carcinogenesis and of lipid peroxidation by tea catechins, which are naturally occurring polyphenols, have been reported.1−5) These effects are regarded as being due to the antioxidative property of catechins. We have also reported that, in the presence of the copper(II) ion under aerobic conditions, tea catechins induced DNA cleavage,6) accelerated the peroxidation of unsaturated fatty acid,7) and killed Escherichia coli.7) These effects were due to the apparent prooxidative property of catechins. We found that these DNA cleavage activities varied with the structure of catechins,8) (−)-epigallocatechin being the most effective.

Except for Cu2+, there has been no report examining the prooxidative activities of catechins toward DNA cleavage under the influence of various metal ions. In this study, we examine the activities of 4 catechins toward DNA cleavage with 16 metal ions: Ca2+, Fe3+ and Mg2+ which exist in large amounts in the living body, and Ag+, Al3+, Cd2+, Co2+, Cr3+, Cu2+, Mn2+, Mo6+, Ni2+, Pb2+, Se4+, Sn2+ and Zn2+ which exist in small amounts. (−)-Catechin (C), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (EGCg) were used (Fig. 1).

All the reagents used for the experiments, other than DNA, were purchased from Wako Co. (Japan). A reaction solution to examine the DNA cleavage was prepared by dissolving a catechin and metal ion in water. Calf thymus DNA from Worthington Biochemical Co. (U.S.A.) was dissolved in a sodium phosphate buffer (72.5 mM, pH 7.2), the concentration of the DNA stock solution being 0.2 mg of DNA/ml. The DNA stock solution (200 μl) and the catechin solution (50 μl) were mixed, and then the solution of the metal ion (10 μl) was added. The total volume was adjusted to 400 μl with the sodium phosphate buffer (72.5 mM, pH 7.2). The final concentrations of DNA, catechin, and the metal ion

![Structures of the Tea Catechins](image-url)
were 40 μg/400 μl, 0-9 × 10⁻⁴ M, and 0-5 × 10⁻⁵ M, respectively. The mixture was allowed to react at 37°C under aerobic conditions. After 1 hour, the amount of remaining DNA was analyzed by the fluorescence method with ethidium bromide.⁹³

As summarized in Fig. 2, only Cu⁺² and Ag⁺ showed a synergistic effect with the catechins on DNA cleavage, while the other metal ions examined, Al³⁺, Ca²⁺, Cd²⁺, Co³⁺, Cr³⁺, Fe³⁺, Mg²⁺, Mn²⁺, Mo⁶⁺, Ni²⁺, Pb²⁺, Se⁴⁺, Sn⁺² and Zn⁺², showed little effect, the normal effect of each metal ion itself being retarded. The copper(II) ion by itself rarely cleaves DNA, but Cu⁺² with a catechin facilitated DNA cleavage (Fig. 3). The order of activity of the catechins to the DNA cleavage was EGC > EC > EGCG > C in the presence of Cu⁺². It is remarkable that EC was more active than C, although the difference was only due to the opposite stereochemistry at C-3 of the C-ring. Ag⁺ by itself, however, was able to cleave DNA at a concentration of 5.0 × 10⁻³ M Ag⁺, although not at 5.0 × 10⁻⁵ M Ag⁺. The presence of Ag⁺ with either EGC or EC (2.4 × 10⁻⁴ M) showed a strong repressive effect on DNA cleavage with 5.0 × 10⁻³ M Ag⁺ (Fig. 4). It is interesting that the catechin activity order was apparently opposite with Cu⁺² and Ag⁺, the order for the repressive effect by the catechins on DNA cleavage by Ag⁺ being EGC > EC > EGCG. As shown in Fig. 5, 83% of DNA was cleaved by 5.0 × 10⁻³ M of Ag⁺, DNA cleavage was retarded to

![Figure 2](image-url)  
**Fig. 2.** Effects of Catechins on DNA Cleavage in the Presence of Various Kinds of Metal Ions.

DNA (40 μg) was incubated with a catechin (2.4 × 10⁻⁴ M) and metal ion (5.0 × 10⁻³ M) in a 72.5 mM sodium phosphate buffer at pH 7.2 and 37°C for 1 h under aerobic conditions.  
- Metal ion with EGC,  
- Metal ion with EC,  
- Metal ion with EGCG,  
- Metal ion only.

![Figure 3](image-url)  
**Fig. 3.** Effects of Catechins on DNA Cleavage in the Presence of Cu⁺².

DNA (100 μg) was incubated with various concentrations of a catechin (0-9.0 × 10⁻⁴ M) and Cu⁺² (6.8 × 10⁻³ M) in a 72.5 mM sodium phosphate buffer at pH 7.2 and 37°C for 1 h under aerobic conditions.
- Cu⁺² with EGC,  
- Cu⁺² with EC,  
- Cu⁺² with EGCG,  
- Cu⁺² with C.

![Figure 4](image-url)  
**Fig. 4.** Effects of Catechins on DNA Cleavage in the Presence of Ag⁺.

DNA (40 μg) was incubated with a catechin (2.4 × 10⁻⁴ M) and various concentrations of Ag⁺ (0-5.0 × 10⁻³ M) in a 72.5 mM sodium phosphate buffer at pH 7.2 and 37°C for 1 h under aerobic conditions.  
- Ag⁺ with EGC,  
- Ag⁺ with EC,  
- Ag⁺ with EGCG,  
- Ag⁺ only.
Fig. 5. Effects of Catechins on DNA Cleavage in the Presence of Ag⁺.

DNA (40 μg) was incubated with various concentrations of a catechin (0-6.4 × 10⁻⁴ M) and Ag⁺ (5.0 × 10⁻⁵ M) in a 72.5 mM sodium phosphate buffer at pH 7.2 and 37°C for 1 h under aerobic conditions. ○ Ag⁺ with EGC, ● Ag⁺ with EC, ○ Ag⁺ with EGCg.

25% by the addition of 2.5 × 10⁻⁴ M of EGC or 3.75 × 10⁻⁴ M of EC, while EGCg (6.25 × 10⁻⁴ M) with Ag⁺ (5.0 × 10⁻⁵ M) did not repress DNA cleavage.

These results must be related to the redox potentials of catechins, metal ions and oxygen, and to the reversibility of redox reactions with the metal ions under the experimental conditions. Further study is in progress in this respect.

References