12.14 Protecting Effect on Gamma-ray Damage of DNA by Tea Catechin

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Abstract

The protecting effect of the tea catechin on the radiation induced scission of DNA *in vitro.* was examined. In addition, ESR spin-trapping method was used to make clear the mechanism of the protection.

INTRODUCTION

Recently, it has been shown *in vivo* and *in vitro* [1),2)] that tea catechin suppress mutation and carcinogenesis. Main components of the catechin is (-)-epigallocatechin gallate(EGCg). The content of EGCg exceeds 50% of the total catechin and show in many cases the strongest activity of the suppression on the mutation and the carcinogenesis. However, the mechanism is not sufficiently clarified. It is well known that carcinogenesis, mutation and aging include the chemical process of DNA scission and the DNA scission is induced by radiation as well as chemical carcinogenesis. Therefore, we examined quantitatively the protecting effect of the tea percolate and EGCg on the radiation induced scission of DNA *in vitro.* In addition, we tried to make clear the mechanism of the protection using ESR spin-trapping method.

EXPERIMENTAL

Preparation of the Samples and the Reagent: Plasmid pUC18 DNA was prepared from *E.coli by a* conventional method. EGCg(WAKO, guaranteed reagent) was dissolved into SSC buffer solution(0.15M NaCl, 0.015M Na-citrate, pH7) to be $1x10^{-1}$ M just before the experiment, then diluted to the required concentration. Specially purified spin trapping agent, 5,5-dimethyl-1-pyrroline N-oxide (DMPO), was obtained from

LABOTEC Co. Ltd. All other reagents used are guaranteed reagents.

Apparatus: Gamma irradiation was carried out with ^{60}Co source at Shizuoka University. Radiation doses at various points from the source were measured by Fricke dosimeter. Mini-gel electrophoresis system (Mupid, ADVANE Co. Ltd.) and densitometer (Atto-DCD-16) were used. ESR spectra were measured by JEOL RE3X spectrometer (X-band) with 100 kHz field modulation.

Procedure: (1) 4 μ l of SSC buffer solutions of DNA (0.019 μ g/ μ l) were placed in micro-tubes. (2) To them, 6 μ l of tea percolate or SSC buffer solutions containing various amounts of EGCg were added. (3)Those samples were irradiated with ^{60}Co source for 2h. (4) After finishing irradiation, DNA was separated to each forms explained later by electrophoresis. (5)Each bands stained by etidium bromide were photographed. (6)Degree of the darkening of the bands on the film were measured by a densitometer and the integrated area were calculated with a computer. (7) Survival percentage of CCC form DNA used as a index of the protecting effect was calculated with the following equation.

$$\text{Survival (\%)} = \frac{1.42 \times [\text{CCC}]}{1.42 \times [\text{CCC}] + [\text{OC}] + [\text{L}]} \times 100$$

Here, L means linear form DNA formed by the double strand scission, but it was not detected in this experiment. The values in the brackets mean the degree of darkening and the factor, 1.42, is a correction coefficient for CCC form.

Spin trapping experiments were carried out as follows. (1)75 μ l of aqueous $FeCl_2$ solutions were placed in a number of micro-tubes, then 15 μ l of DMPO were added. (2)Next, 35 μ l of water or SSC buffer or 35 μ l of the solutions containing the protecting agents were added and vortexed for mixing. (3)75 μ l of H_2O_2 solutions(1×10^{-3} M) were added and mixed quickly. Time was measured from the mixing. (4)The mixtures were sucked into capillary glass tubes (Drummond 75 μ l micropipette, 0.8 mm i.d.) and the one end of the tubes was sealed with teflon tubes and ERS spectra were measured.

RESULTS AND DISCUSION

Protection by Tea Percolate Fig. 1 shows the protecting effect of green tea percolate. The photograph is the electrophoresis pattern showing native covalently closed circular (CCC) and open circular (OC) form DNA. Sample 5 is the control, which was non-irradiated, not containing tea percolate, contains only CCC form. As shown in the sample 4, CCC form DNA was decomposed perfectly into OC form under this irradiation condition. However, CCC form still remains in the samples 1 ~ 3 and the survival ratios depend on the quantities of the tea percolate. In the samples containing the same amounts of tea percolate as samples 1 ~ 4 and having been stood for 2 h without irradiation, DNA scission was not observed, showing that tea percolate really protected irradiation-induced scission. The survival (%) were tabulated in Table 1.

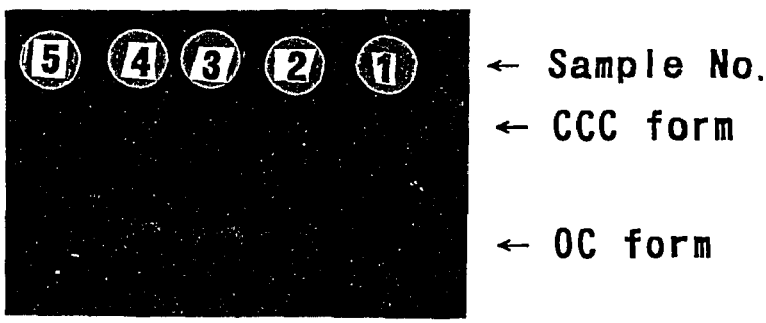

Fig. 1 Electrophoresis pattern of γ -ray irradiated DNA.

Table 1 Protecting effect of green tea percolate on DNA scission by γ ray

Sample No	Relative cocentration of tea percolate *	CCC DNA survival (%)
1	1.00	95.3 (irrad.)
2	0.1	77.0 (irrad.)
3	0.01	45.2 (irrad.)
4	0	0 .0 (irrad.)
5 (control)	0	100 .0 (not irrad.)

Green tea (4g) was percolated with hot water (75 °C, 50 ml, 3 min). Sample 1 is the original percolated solution, and 2 and 3 are diluted 10 and 100 times of the original.

Protection by EGCg. Fig. 2 shows the protecting effect of EGCg and the dependency on the EGCg concentration and the radiation dose. The protecting effect increased with

increase in the EGCg concentration. But, DNA scission increased with increase in the radiation dose in all the samples containing different amounts of EGCg.

Duration of the Protecting Effect of EGCg. EGCg is susceptible to the autoxidation even at the room temperature and browning is observed while it is stood as aqueous solutions for a few days. Therefore, it is a problem whether the protecting effect is kept while standing. To ascertain it, we compared the protecting effect of a freshly prepared EGCg solution and the solution stood for a day at room temperature. Difference was not observed between them, showing that the effect was kept at least for a day.

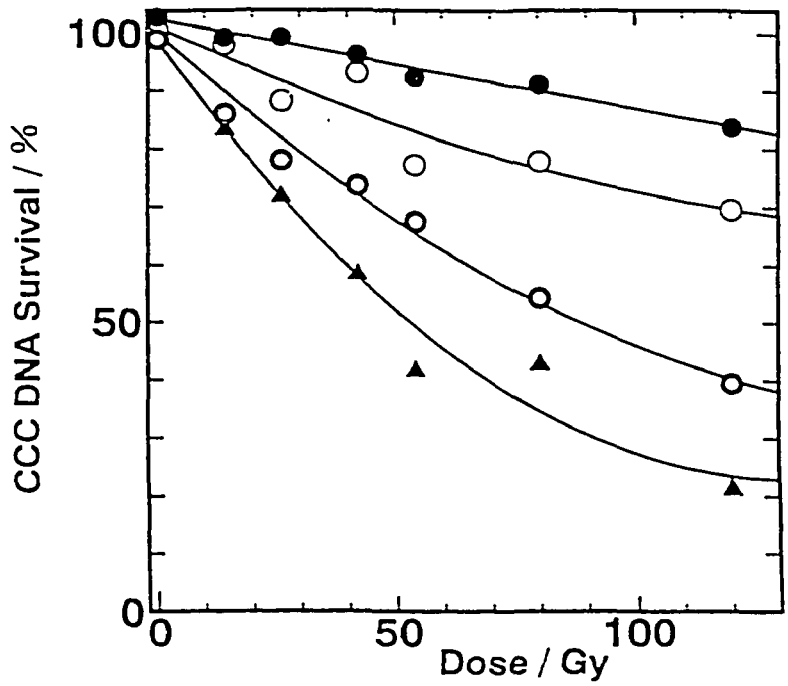

Fig. 2 Dependence of CCC Survival on the radiation dose under various concentrations of EGCg. (DNA) : 0.019 μg / μl, Irradiation time : 2h
▲ 0 M, ◎ 3×10^{-4} M, ○ 1×10^{-3} M, ● 3×10^{-3} M

Spin Trapping Experiment. It is generally accepted that the radiation-induced scission of DNA is caused mainly by OH radical formed from water molecule. For example, t-butanol known as a scavenger of OH radical suppressed DNA scission. Therefore, it is reasonable to consider that the protecting effect of EGCg is attributed to the scavenging effect of OH radical. We examined the OH radical scavenging activity of EGCg. Here, OH radical was generated by Fenton reaction and was trapped with

DMPO. Fig. 3 shows the results, where the intensity of the spin adduct, DMPO-OH, was decreased when EGCg was coexistent, suggesting that EGCg acts as the scavenger.

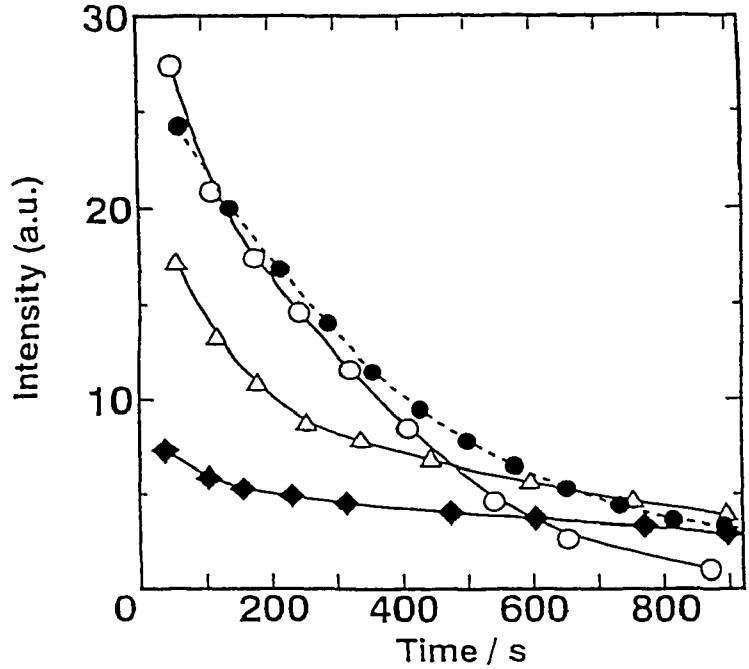

Fig. 3 ESR intensity of DMPO-OH under various EGCg concentrations
○ 1×10^{-4} M, △ 1×10^{-3} M, ◆ 1×10^{-2} M, ● 0 M

CONCLUSIONS

1) Tea percolate and the main ingredient EGCg suppress the radiation-induced scission of DNA.
2) Spin-trapping method showed that EGCg acted possibly as a OH radical scavenger.
3) Protecting effect of EGCg on the radiation-induced scission of DNA is attributable to the OH radical scavenging activity.

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