DNA AND RNA AS NEW BINDING TARGETS OF GREEN TEA CATECHINS*

Takashi Kuzuhara 1, Yoshihisa Sei2, Kentaro Yamaguchi2, Masami Suganuma3 and Hirota Fujiki1

From 1. Laboratory of Biochemistry, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan
2. Analytical Chemistry Laboratory, Faculty of Pharmaceutical Sciences at Kagawa Campus, Tokushima Bunri University, Sanuki-city, Kagawa 769-2193, Japan
3. Research Institute for Clinical Oncology, Saitama Cancer Center, Kitaadachi-gun, Saitama 362-0806, Japan

Address Correspondence to: Takashi Kuzuhara, Laboratory of Biochemistry, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima, 770-8514, Japan, Tel. 81-88-622-9611; Fax. 81-88-655-3051; E-Mail: kuzuhara@ph.bunri-u.ac.jp

The significance of catechins, the main constituents of green tea, is being increasingly recognized with regard to cancer prevention. Catechins have been studied for interactions with various proteins, but the mechanisms of the various catechins are not yet elucidated. Based on our previous observation that nucleic acids extracted from catechin-treated cells are colored, we studied whether catechins directly interact with nucleic acids using surface plasmon resonance assay (Biacore) and cold spray ionization (CSI) mass spectrometry (MS). These two methods clearly showed that (-)-epigallocatechin gallate (EGCG) binds to both DNA and RNA molecules: the Biacore assay indicated that four catechins bound to DNA oligomers, and CSI-MS analysis showed one to three EGCG molecules bound to single strand 18 mers of DNA and RNA. Moreover, one or two molecules of EGCG bound to double-stranded AG: CT oligomers of various nucleotide lengths. These results suggest that multiple binding sites of EGCG are present in DNA and RNA oligomers. Double-stranded DNA oligomers were detected only as EGCG-bound forms at high temperature, whereas at low temperature both the free and bound forms were detected, suggesting that EGCG protects dsDNA oligomers from dsDNA-melting to ssDNA. Since both galloyl and catechol groups of EGCG are essential for DNA binding, both groups seem to hold strands of DNA via their branching structure. These findings reveal for the first time the link between catechins and polynucleotide, and will intensify our understanding the effects of catechins on DNA in terms of cancer prevention.

Green tea is an acknowledged cancer preventive in Japan (1-7). Most of the active principles are assumed to be green tea catechins, because they show various cancer preventive activities in vitro, in cell culture, and in vivo, including anti-oxidant, anti-cancer, and anti-mutagenic activities (1-7). (-)-Epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epicatechin (EC), (-)-gallocatechin gallate (GCG) and (+)-catechin are the major components of green tea polyphenols (8), and ECGC is the main constituent of green tea (9). Since we first reported in 1987 that topical applications of EGCG inhibited tumor promotion with 12-O-
tetradecanoylphorbol-13-acetate (TPA) and okadaic acid on mouse skin in two-stage carcinogenesis experiments (10, 11), we have studied the mechanisms of action of green tea catechins in human cancer cell lines (12-18). During our study of EGCG, we often observed that nucleic acids extracted from EGCG-treated human cancer cells were catechin-colored, suggesting that EGCG binds to DNA and RNA molecules in cells. Our speculation was strengthened by results showing that 3H-EGCG was found in nuclei of human lung cancer cell line PC-9 1 hr after treatment (13). While EGCG apparently protects against DNA damage induced by free radicals, ionization and ultraviolet radiation, as well as DNA methylation (5, 19, 20), it was not yet shown whether EGCG and other green tea catechins directly bind to DNA and RNA molecules. Since we had demonstrated that EGCG inhibited expression of the tumor necrosis factor-α (TNF-α) gene in human cancer cells treated with the tumor promoter okadaic acid (12, 16), we looked at the effects of EGCG on the expression of 588 genes in PC-9 cells, using a human cancer cDNA expression array: Results showed up regulation of four genes (at least 2.0 fold) and down regulation of 12 genes (under 0.5 fold) (1, 21). It has also been reported that catechins affect DNA replication, DNA repair and transcription (20-25), and that all these effects can be explained through the actions of catechin on enzymes (20-26). Thus, our new research objective was to find out whether EGCG directly interacts with DNA and RNA molecules.

Surface plasmon resonance (Biacore) assay is a real-time analysis that was developed as a methodology for determining molecular interaction under aqueous conditions. Cold spray ionizing (CSI) - mass spectrometry (MS), a variant of electrospray ionization (ESI) - MS developed by one of the authors - has numerous advantages in determining molecular interaction based on the forming molecular ion in solution (27). Using these two new technologies, we demonstrated for the first time the direct binding of EGCG to DNA and RNA oligomers and investigated the structure-function relationship of this interaction. The results will likely provide a new knowledge of the molecular mechanisms of action of green tea catechins, and show that green tea catechins have multifunctional targets for the prevention of human cancer.

Experimental procedures

**Materials** - EGCG was purified from Japanese green tea leaves with 99.7% purity. EGCG, EGC, ECG, EC, gallic acid (GA), GCG, (-)-catechin gallate (CG), (-)-gallocatechin (GC), (+)-catechin (C) and theaflavin were purchased from Funakoshi Co. Ltd. (Tokyo, Japan). DNA and RNA oligomers were synthesized, and further purified by High Performance Liquid Chromatography. Their purities were examined using polyacrylamide gel electrophoresis and TOF-MASS by Hokkaido System Science Co., Ltd. Double stranded DNAs (dsDNAs) were made by annealing complementary single strand DNAs (ssDNAs) in 100 mM ammonium acetate. The DNA and RNA oligomers used were as follows: poly dA18, poly dT18, poly dC18, poly dG18, poly A18, poly U18, poly G18, poly C18, poly dA9dT9 and poly dG9dC9. In addition, dsDNA oligomers of dAdC: dTdG from six to fourteen nucleotides were also used (28). For Biacore assay, 20mers of oligo DNA biotinylated at 5' end were immobilized on sensor chip SA (streptavidine).

**Surface Plasmon Resonance Assay (Biacore Assay)** - Biacore 3000 (Biacore AB, Uppsala, Sweden) was used for surface plasmon resonance analysis, according to the manufactures' instructions. The methods with buffer can be described briefly as follows: We used sensor chip SA and HBS-N buffer (10 mM HEPES pH 7.4, 150 mM NaCl) as running buffer. 10 µl of 2 µM biotinylated DNA oligomers were immobilized to sensor
EGCG and other catechins were originally dissolved in methanol at 10 mM as stock solution. At various concentrations (1.6, 3.3, 6.5, 13, 25 and 50 µM) in HBS-N buffer, they were applied to the immobilized ssDNA and dsDNA at 20 µl/min as flow rate. Blank was used as reference and was subtracted from all the raw data. For the affinity analysis, we used BIAevaluation steady state affinity software. The results with 10 µM EGCG and other catechins are presented in Figure 2.

Cold Spray Ionization (CSI) Mass Spectrometry - 0.1 mM EGCG and 0.05 mM DNA, or 0.1 mM EGCG and 0.05 mM RNA were mixed in 100 µl of 100 mM ammonium acetate and then injected into CSI mass spectrometry (27). For CSI mass spectrometry, high concentrations of molecules are necessary for detection. For binding analysis of dsDNA, annealed poly dA3dG3 + poly dC3dT3, poly dA4dG4 + poly dC4dT4, poly dA5dG5 + poly dC5dT5, poly dA6dG6 + poly dC6dT6, poly dA7dG7 + poly dC7dT7 were used (28). Mass spectral measurements were performed with sector (BE) mass spectrometer (JMS-700, JEOL) equipped with a cold-spray ionization (CSI) source. Experimental conditions were as follows: experimental (negative), acceleration voltage: 5.0 kV, needle voltage: -3.5 kV, orifice voltage: -73 V, ring lens voltage: -132 V, spray temp: room temperature, resolution: 1000, flow rate: 0.5 ml/hr, solvent: H2O.

RESULTS

Interaction Between DNA oligomers and EGCG determined by Biacore Assay, and Their Structure-Function Relationship - We tested the interaction between catechins and DNA using two different methods: Biacore analysis, which indicates the real time binding, and CSI-MS, which shows the endpoint binding. The Biacore assay method makes it possible to observe the association and subsequent dissociation of DNA with EGCG in real time. We analyzed the interaction between EGCG and poly dT 20 mer oligo ssDNA at various concentrations (1.6, 3.3, 6.5, 13, 25 and 50 µM) of EGCG (Fig. 1). Figure 1 shows both the association, represented by an increase in resonance unit, and the dissociation, represented by a decrease in resonance unit. Poly dT ssDNA oligomers immediately associated with EGCG and rapidly dissociated from it. The resonance units of EGCG to DNA increased dose-dependently (red for 1.6 µM dark, purple for 3.3 µM, green for 6.5 µM, blue for 13 µM, dark blue for 25 µM and brown for 50 µM EGCG) (Fig. 1A). This is the first demonstration that EGCG directly binds to DNA. For the binding parameters, we conducted steady state affinity analysis using BIAevaluation software. The Kd value was estimated to be 5.4 x 10^-5 M (Fig. 1B). The effective concentrations of EGCG for growth inhibition of a human lung cancer cell line are almost the same as this Kd value (3), and EGCG subsequently accumulated in the cells. Thus we think that the Kd value 5.4 x 10^-5 M is reasonable (for more details, see Discussion).

The binding curves of EGCG with poly dA (red line), poly dT (blue line) and poly dG (red line) DNA oligomers are shown in Figure 2a. (The results with poly dC are not shown). Poly dT oligomer bound to EGCG, and poly dA and dG oligomers showed weak binding to EGCG, indicating the presence of base–preference for EGCG-DNA-binding. Since ethidium derivatives are known to bind to AT-rich sequences via a minor groove, EGCG may recognize a higher-order structure of DNA.

To study the structure-function relationship of EGCG for DNA-binding, we next determined which chemical group of EGCG is important for the interaction with DNA. We used several catechins and related chemicals: EGCG, ECG, EGC, EC, GA, GCG, CG, GC, C and theaflavin. In addition to EGCG, ECG, GCG and CG also showed the association with poly dT 18 mer (Fig. 2a, b,
g, and h). Other catechins, such as EGC, EC, GC, C and theaflavin, did not interact with DNA oligomers (Fig. 2c, d, i, j and k). Based on our results, we think that the binding to DNA oligomers is not influenced by the (-)-epi- or non (-)-epi forms of catechins. Next, we designed a putative tertiary structure of various catechins and used them for binding analysis (Fig. 2). The structure-function relationship of catechins and DNA oligomers indicated the significance of the branching structure, consisting of galloyl and catechol groups (see the putative molecular models of the tertiary structures in Fig. 2 right column). To confirm the usefulness of the branching structure, a mixture of EC and GA were tested, but no association was observed (Fig. 2f and l): This suggests an insufficient interaction when the two chemical groups are separated, but strengthened the importance of these two groups when linked by covalent bond. Although the exact binding site and the mode of interaction between catechins and DNA have not been resolved yet, we assume that the branching structure of EGCG is attached to some parts of DNA molecule. All the results strongly encouraged us to pursue further experiments with DNA oligomers and EGCG using the CSI - MS analysis.

Multiple EGCG-binding sites in DNA Oligomers determined by CSI - MS Analysis - Mass spectrometry can show the exact molecular ratio of binding molecules. CSI - MS is a direct analysis method that promotes electrolytic dissociation forming the molecular ion in solution at low temperature (27, 28). This method has the advantage of detecting extremely labile complexes of biological molecules without causing decomposition. For the experiments, 50 µM DNA oligomers - such as single stranded 18 mers of poly dA, poly dT, poly dG, poly dC, poly dAdT and poly dGdC were mixed with 0.1 mM EGCG. For example, the molecular weights of poly dT 18 mer and EGCG are 5,414 and 458, respectively. In the CSI - MS analysis, the molecular weights are presented as numbers divided by the charge numbers. Various peaks of poly dT 18 mer and the bound form, DNA + EGCG, appear in Figure 3. As an example, peaks of [DNA]^{4+} and [DNA + 2EGCG]^{4+} were output in the m/z ranges of 1350.3 and 1579.0, respectively. The number 4 represents the charge number of molecules in the ionization in this case; when the difference between the two values is multiplied by four, resulting in 915, the value corresponds to the molecular weight of two molecules of EGCG, which is 916. These results indicate that two molecules of EGCG directly associated with DNA. Other peaks were also calculated and identified as shown in Figure 3. Specifically, three kinds of bound forms, (DNA + EGCG), (DNA + 2EGCG), and (DNA + 3EGCG), were observed, showing that at least three molecules of EGCG bind to poly dT 18 mer, and suggesting that multiple binding sites for EGCG are present in DNA oligomer (Table 1).

To study the nucleotide specificity of DNA oligomer for EGCG-binding, the next experiments were conducted with 18 mers of poly dA, dG and dC, and 9 mers of dAdT and dGdC. The results are summarized in Table 1. While poly dT oligomers bound to 3 molecules of EGCG, poly dA, poly dC and poly dAdT showed weak binding to at least one molecule of EGCG, which corresponds to the results of the Biacore assay. No interaction between poly dG 18 mer and EGCG was detected, probably due to difficulty of ionization in the assay conditions. All the results in CSI-MS analysis showed that EGCG binds to ssDNA oligomers. The question of why three molecules of catechin, but not more, bind to the 18-mers will be discussed in the next part.

Interaction Between Single Strand RNA (ssRNA) Oligomers and EGCG determined by CSI - MS Analysis - Based on evidence that ssDNA oligomers directly bind to EGCG, we next tested the binding of ssRNA oligomers by CSI - MS analysis. For this experiment, 0.5 mM 18 mers of poly A, U,
G and C, and 18 mers of AU and GC, and 0.1 mM EGCG were mixed. Figure 4 shows three bound forms of EGCG, (RNA + EGCG), (RNA + 2EGCG), and (RNA + 3EGCG), suggesting that poly A 18 mer binds to at least 3 molecules of EGCG (Table 1). In addition, the 18 mer of poly AU bound to one molecule of EGCG, indicating that poly A, poly U and poly AU also bind to EGCG. However, the interaction between poly G and EGCG was not clearly observed, in contrast to the interaction between poly dG and EGCG. This suggests that oligomers of poly G and poly dG do not readily ionize under these assay conditions. Since EGCG binds to both ssDNA and ssRNA oligomers, we assume that the 2’ hydroxyl group of ribose does not influence the interaction with EGCG.

Interaction Between dsDNA Oligomers and EGCG, Determined by CSI - MS Analysis and Biacore Assay - It is necessary to study the interaction between dsDNA and EGCG more precisely, since DNA exists as a double strand in the genome. As in the case of ssDNA, we conducted two different assays for this subject. Initially, various lengths of double stranded AG:CT oligomers (from 6 to 14 base pairs) were mixed with EGCG under identical conditions. Figure 5 shows that one or two molecules of EGCG bound to all double stranded AG:CT oligomers, without any relation to nucleotide length.

To study the binding mode of EGCG and dsDNA, the assay was conducted at high (48 ºC) and low temperatures (28 ºC), using six base oligomer dsDNAs (Fig. 6). As Figure 6A shows, dsDNA oligomers were detected only as EGCG-bound forms at high temperature, although at low temperature both the free and EGCG-bound forms were present. At the high temperature this length of oligomer dsDNA normally melts into ssDNA. These data suggest that EGCG protects dsDNA oligomers from dsDNA-melting to ssDNA.

In the Biacore assay, we detected the association of EGCG with the annealed 20 mer DNA (Fig. 7a), but no association with other catechins was found (Fig. 7b-d). Judging from these different experiments, we conclude that EGCG also binds to dsDNA. However, the other catechins did not, which indicates that one hydroxyl group of the trihydroxyphenyl group in EGCG is essential for binding to dsDNA. The galloyl group is also essential for binding to both ssDNA and dsDNA. The association-dissociation curve is different in the experiments between EGCG-ssDNA and EGCG-dsDNA, and the difference probably reflects the binding mode of EGCG to each DNA.

DISCUSSION

Based on numerous reports, the interaction of EGCG with various proteins and lipids is now widely accepted. Catechins affect reactions associated with DNA, and in those cases EGCG is thought to interact with enzymes on DNA molecules (20-25). This is the first experiment demonstrating a direct interaction between catechin and polynucleotides, so, this paper reports a new molecular mechanism of action for green tea catechins.

We previously reported that duplicate 3H-EGCG administrations at 6 h interval enhanced incorporation of 3H-EGCG 4 to 9 times in most organs compared with a single administration. Since this enhancement showed that EGCG accumulates in cells, we named it the “Fujiki-Suganuma Effect” (29, 30). Although it is not clear yet which molecules promote EGCG accumulation in cells, the results of the current experiment allow us to assume that both DNA and RNA molecules can act as biological reservoirs for EGCG. In most experiments in cell lines treated with EGCG, the concentrations of EGCG are relatively higher than those of the usual active compounds, but we think such concentrations are significant in vivo: EGCG and green tea catechins accumulate in the whole body by consumption of green tea throughout the day.

It is of interest to note why more than three molecules of catechin were not found
on the 18 mers and, there are at least two possible explanations. One is the limitation in the sensitivity of mass spectrometry: The complex of ssDNA with more than three molecules of EGCG would be broken upon ionization of CSI, and a peak of the complex of ssDNA with more than three molecules of EGCG would be covered by the background peaks. Another possibility is that the binding surface is limited because of the tertiary structure of ssDNA, which influences the interaction. This problem can be resolved by NMR or x-ray crystallography.

Recently, administration of green tea was reported to alter the mutation profile of p53 (31), and our findings here on the binding of catechins to DNA support this change in the mutation profile of DNA. Catechin inhibits the activities of various proteins attached to DNA, such as DNA polymerase, CRE-binding protein, DNA methyltransferase, and DNA topoisomerase (21-26). These reactions are likely affected by EGCG-binding to DNA and RNA.

Catechins induce apoptosis in cells and sometimes cause DNA damage (21-26), phenomena that are due to oxidative stress. However, this might be triggered by the direct binding of catechins to DNA.

Judging from our findings, the mechanisms of anti-cancer and anti-aging activities of catechins are intensified through the binding of catechins to DNA and RNA.

REFERENCES

1. Fujiki, H., and Suganuma, M. (2002) *Proc. Japan Acad.* **78**(B), 263-270
2. Fujiki, H., Suganuma, M., Imai, K., and Nakachi, K. (2002) *Cancer Lett.* **188**, 9-13
3. Fujiki, H. (2005) *Chem. Rec.* **5**, 119-132
4. Nakachi, K., Matsuyama, S., Miyake, S., Suganuma, M., and Imai, K. (2000) *BioFactors* **13**, 49-54
5. NCI, DCPC. (1996) *J. Cell Biochem.* **26**S, 236–257
6. Yang, C. S., and Wang, Z. -Y. (1993) *J. Natl. Cancer Inst.* **85**, 1038–1049
7. Wang, Z. Y., Hong, J. Y., Huang, M. T., Reuhl, K. R., Conney, A. H., and Yang, C. S. (1992) *Cancer Res.* **52**, 1943-1947
8. Okuda, T., Mori, K., and Hatano, T. (1985) *Chem. Pharm. Bull. (Tokyo)* **33**, 1424-1433
9. Fujiki, H., and Okuda, T. (1992) *Drugs of the Future* **17**, 462-464
10. Yoshizawa, S., Horiuchi, T., Fujiki, H., Yoshida, T., Okuda, T., and Sugimura, T. (1987) *Phytother. Res.* **1**, 44-47
11. Yoshizawa, S., Horiuchi, T., Suganuma, M., Nishiwaki, S., Yatsunami, J., Okabe, S., Okuda, T., Muto, Y., Frenkel, K., Troll, W., and Fujiki, H. (1992) *ACS Symposium Series* **507**, 316-325
12. Suganuma, M., Okabe, S., Sueoka, E., Iida, N., Komori, A., Kim, S. J., and Fujiki, H. (1996) *Cancer Res.* **56**, 3711-3715
13. Okabe, S., Suganuma, M., Hayashi, M., Sueoka, E., Komori, A., and Fujiki, H. (1997) *Jpn. J. Cancer Res.* **88**, 639-643
14. Kitano, K., Nam, K. Y., Kimura, S., Fujiki, H., and Imanishi, Y. (1997) *Biophys. Chem.* **65**, 157-164
15. Okabe, S., Ochiai, Y., Aida, M., Park, K., Kim, S. J., Nomura, T., Suganuma, M., and Fujiki, H. (1999) *Jpn J. Cancer Res.* **90**, 733-739
16. Suganuma, M., Sueoka, E., Sueoka, N., Okabe, S., and Fujiki, H. (2000) *BioFactors* **12**, 1–6
17. Sueoka, N., Suganuma, M., Sueoka, E., Okabe, S., Matsuyama, S., Imai, K., Nakachi, K., and Fujiki, H. (2001) *Ann. N. Y. Acad. Sci.* **928**, 274-280
18. Fujimoto, N., Sueoka, N., Sueoka, E., Okabe, S., Suganuma, M., Harada, M., and Fujiki, H. (2002) *Int. J. Oncol.* **20**, 1233-1239
19. Fujiki, H. (1999) *J. Cancer Res. Clin. Oncol.* **125**, 589-597
20. Fang, M. Z., Wang, Y., Ai, N., Hou, Z., Sun, Y., Lu, H., Welsh, W., and Yang, C. S. (2003) *Cancer Res.* **63**, 7563-7570
21. Okabe, S., Fujimoto, N., Sueoka, N., Suganuma, M., and Fujiki, H. (2001) *Biol. Pharm. Bull.* **24**, 883-886
22. Johnson, M. K., and Loo, G. (2000) *Mutat. Res.* **459**, 211-218
23. Berger, S. J., Gupta, S., Belfi, C. A., Gosky, D. M., and Mukhtar, H. (2001) *Biochem. Biophys. Res. Commun.* **288**, 101-105
24. Bertram, B., Bollow, U., Rajaee-Behbahani, N., Burkle, A., and Schmezer, P. (2003) *Mutat. Res.* **534**, 77-84
25. Balasubramanian, S., and Eckert, R. L. (2004) *J. Biol. Chem.* **279**, 24007-24014
26. Mizushina, Y., Saito, A., Tanaka, A., Nakajima, N., Kuriyama, I., Takemura, M., Takeuchi, T., Sugawara, F., and Yoshida, H. (2005) *Biochem. Biophys. Res. Commun.* **333**, 101-109
27. Yamaguchi, K. (2003) *J. Mass Spectrom.* **38**, 473-490
28. Sakamoto, S., and Yamaguchi, K. (2003) *Angew. Chem. Int. Ed. Engl.* **42**, 905-908
29. Suganuma, M., Okabe, S., Oniyama, M., Tada, Y., Ito, H., and Fujiki, H. (1998) *Carcinogenesis* **19**, 1771-1776
30. Fujiki, H., and Suganuma, M. (2005) *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev.* **23**, 3-30
31. Kramata, P., Lu, Y. P., Lou, Y. R., Cohen, J. L., Olcha, M., Liu, S., and Conney, A. H. (2005) *Carcinogenesis* **26**, 1965-1974

FOOTNOTES

* We thank Dr. Marsh R. Rosner for critical reading the manuscript. We also thank Dr. Midori Suenaga for fruitful discussion and Ms. Mitsuyo Kato and Ms. Kayoko Nagata for their technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan and the Smoking Research Fund.

1The abbreviations used are: CSI, cold spray ionization; MS, mass spectrometry; EGCG, (-)-epigallocatechin gallate; EGC, (-)-epigallocatechin; ECG, (−)-epicatechin gallate; EC, (−)-epicatechin; GCG, (−)-gallocatechin gallate; GA, gallic acid; CG, (−)-catechin gallate; GC, (−)-gallocatechin; C, (+)-catechin; TPA, 12-O-tetradecanoylphorbol-13-acetate; TNF-α, tumor necrosis factor-α; dsDNA, double stranded DNA; ssDNA, single strand DNA; SA, streptavidine.

FIGURE LEGENDS

Fig. 1. Interaction between DNA oligomer and EGCG determined by surface plasmon analysis (Biscore assay). Biotinized oligomer DNA was immobilized on sensor chip SA. Various concentrations of EGCG were applied to DNA-immobilized sensor chip. Resonance unit reflects the number of attached molecules. A, Red, purple, green, blue, dark blue and brown lines indicate the binding curves of 1.6, 3.3, 6.5, 13, 25 and 50 µM of EGCG with DNA, respectively. The data show resonance unit after subtracting resonance unit of DNA (-) as background from the experimental unit. The resonance units of EGCG to DNA increased dose-
dependently. ‘Association’ and ‘dissociation’ represent the buffer in the presence or absence of the input molecules. B. The affinity analysis of EGCG with DNA, which was conducted based on the data of panel A.

Fig. 2. Interaction between ssDNA oligomer and EGCG, and structure-function-relationship of catechins. Biotinized oligomer DNA was immobilized on sensor chip SA, to which EGCG and various catechins were applied. Resonance unit reflects the number of attached molecules. DNA binding was studied with a) EGCG, b) ECG, c) EGC, d) EC, e) GA, f) EC + GA, g) GCG, h) CG, i) GC, j) C, k) theaflavin, and l) GC + GA. Red, blue and green lines indicate the binding curves of EGCG with poly dA, dT and dG DNA oligomers, respectively. The left column, the middle column and the right column show binding analyses, chemical structures and putative tertiary structures designed by ChemDraw3D™. ‘Association’ and ‘dissociation’ represent the buffer in the presence or absence of the input molecules.

Fig. 3. Direct binding of EGCG to ssDNA oligomers determined by CSI - MS analysis. EGCG and oligomer DNA were mixed and applied to CSI-MS. Peaks are estimated by the indicated molecular weights.

Fig. 4. Direct binding of EGCG to ssRNA oligomers determined by CSI - MS analysis. EGCG and oligomer RNA were mixed and applied to CSI-MS. Peaks are estimated by the indicated molecular weights.

Fig. 5. Direct binding of EGCG to double stranded AG: CT oligomers determined by CSI - MS analysis. Various lengths of dsDNA - A, 14 base pairs (A7G7: C7T7); B, 12 base pairs (A6G6: C6T6); C, 10 base pairs (A5G5: C5T5); D, 8 base pairs (A4G4: C4T4) - were used for the binding experiments.

Fig. 6. Effect of EGCG for melting of dsDNA oligomers. A, CSI-MS analysis of EGCG and 6 base pairs dsDNA (A3G3: C3T3) at high temperature (48 ºC). B, CSI-MS analysis of EGCG and 6 base pairs dsDNA (A3G3: C3T3) at low temperature (28 ºC).

Fig. 7. Structure-function-relationship of catechins for the interaction with dsDNA. Resonance unit reflects attached molecules on sensor chip. a): EGCG, b): ECG, c): EGC, and d): EC. Red, purple and green lines indicate the binding curves of EGCG with poly biotinylated dA;dT, biotinylated dT;dA and biotinylated dG;dC DNA oligomers, respectively.
Table 1 Summary of the interaction of EGCG with various DNA and RNA oligomers

| DNA     | RNA     |
|---------|---------|
| Sequence | Number of bound EGCG | Sequence | Number of bound EGCG |
| dA x 18 | 1       | A x 18   | 3         |
| dT x 18 | 3       | U x 18   | 2         |
| dG x 18 | Not determined | G x 18   | Not determined |
| dC x 18 | 1       | C x 18   | 1         |
| dAdT x 9| 1       | AU x 9   | 1         |
| dGC x 9 | Not determined | GC x 9   | Not determined |

DNA and RNA are synthesized oligomers. The binding of EGCG to dG and G oligomers was not determined because these oligomers rarely ionized in CSI mass spectrometry.
Figure 1

A

B

association
dissociation

RU

Concentration (M)

Resp. Diff.

Time

RU

Concentration

(M)
Figure 2

a) association dissociation

(-)-epigallocatechin gallate (EGCG)

b) association dissociation

(-)-epicatechin gallate (ECG)

c) association dissociation

(-)-epigallocatechin (EGC)

d) association dissociation

(-)-epicatechin (EC)

e) association dissociation

gallic acid (GA)

f) association dissociation

(-)-epicatechin (EC) gallic acid (GA)
Figure 2

(g) association dissociation

(-)-gallocatechin gallate (GCG)

(h) association dissociation

(-)-catechin gallate (CG)

(i) association dissociation

(-)-gallocatechin (GC)

(j) association dissociation

(-)-catechin (C)

(k) association dissociation

theaflavin

(l) association dissociation

(-)-gallocatechin (GC) + gallic acid (GA)
Figure 4

RNA

RNA + EGCG

RNA + 2EGCG

RNA + 3EGCG

1465.6

1431.8

1580.2

1694.8

1809.7

2106.7

2107.4

2259.4
A7G7:C7T7

Figure 5

A

A6G6:C6T6

B
Figure 5

C  A5G5:C5T5

D  A4G4:C4T4
**Figure 6**

A. A3G3:C3T3  high temperature

- ssDNA (A3G3)^{2+}
- ssDNA (C3T3)^{2+}

B. A3G3:C3T3  low temperature

- ssDNA (A3G3)^{2+}
- ssDNA (C3T3)^{2+}
- dsDNA + EGCG
- dsDNA + 2EGCG
- dsDNA + EGCG
- dsDNA + 2EGCG
Figure 7

a) EGCG

b) EC

c) ECG

d) EGC

association
dissociation
DNA and RNA as new binding targets of green tea catechins
Takashi Kuzuhara, Yoshihisa Sei, Kentaro Yamaguchi, Masami Suganuma and Hirota Fujiki

J. Biol. Chem. published online April 25, 2006

Access the most updated version of this article at doi: 10.1074/jbc.M601196200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts