Food Chemistry 217 (2017) 196–204

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Tea polyphenol epigallocatechin gallate inhibits *Escherichia coli* by increasing endogenous oxidative stress

Li-Gui Xiong [a,b,c], Yi-Jun Chen [a], Jie-Wen Tong [a], Jian-An Huang [a,c], Juan Li [a,c], Yu-Shun Gong [a,*], Zhong-Hua Liu [a,b,c,*]

[a] Key Laboratory of Tea Science of Ministry of Education, Hunan Agricultural University, Changsha, Hunan 410128, China
[b] National Research Center of Engineering Technology for Utilization of Functional Ingredients from Botanicals, Hunan Agricultural University, Changsha, Hunan 410128, China
[c] Collaborative Innovation Centre of Utilisation of Functional Ingredients from Botanicals, Hunan Agricultural University, Changsha, Hunan 410128, China

ARTICLE INFO

Article history:
Received 14 April 2016
Received in revised form 12 July 2016
Accepted 25 August 2016
Available online 26 August 2016

Keywords:
Epigallocatechin gallate
Escherichia coli
Reactive oxygen species
Antibacterial
Adaptive response

ABSTRACT

The antibacterial effects of tea polyphenol epigallocatechin gallate (EGCG), a common phytochemical with a number of potential health benefits, are well known. However, the mechanism of its bactericidal action remains unclear. Using *E. coli* as a model organism, it is argued here that H_2O_2 synthesis by EGCG is not attributed to its inhibitory effects. In contrast, the bactericidal action of EGCG was a result of increased intracellular reactive oxygen species and blunted adaptive oxidative stress response in *E. coli* due to the co-administration of antioxidant *N*-acetylcysteine, and not on account of exogenous catalase. Furthermore, we noted a synergistic bactericidal effect for EGCG when combined with paraquat. However, under anaerobic conditions, the inhibitory effect of EGCG was prevented. In conclusion, EGCG caused an increase in endogenous oxidative stress in *E. coli*, thereby inhibiting its growth, and hence the use of EGCG as a prooxidant is supported by this study.

1. Introduction

Green tea (*Camellia sinensis*) has long been associated with many physiological and pharmacological health benefits (Singh, Shankar, & Srivastava, 2011; Yang, Wang, Lu, & Picinich, 2009), although the overall clinical evidence is inconclusive (Singh et al., 2011). Regarded as a functional food and a broad-spectrum botanical, green tea contains many bioactive chemicals such as catechins, including epigallocatechin gallate (EGCG), epicatechin gallate, epigallocatechin and epicatechin (Singh et al., 2011). EGCG, the most abundant polyphenolic catechin in green tea, has been postulated as the principal effective agent (Yang et al., 2009). Consequently, much of the interest in developing nutraceuticals and dietary supplements has focussed on EGCG (Yang et al., 2009).

Over the past two decades, several studies have reported the inhibitory effects of EGCG on both gram-positive and gram-negative bacteria, such as *Enterococcus* spp., *Staphylococcus aureus*, *Streptococcus* spp., *Salmonella* spp., and *E. coli* (Reygaert, 2014; Steinmann, Buer, Pietschmann, & Steinmann, 2013). Its bactericidal effects include damage to the bacterial cell membrane, inhibition of fatty acid synthesis, and inhibition of enzymatic activity (Reygaert, 2014). It is thought that gram-positive bacteria are more susceptible than gram-negative bacteria to EGCG due to the fact that the outer membrane of gram-negative bacteria is mainly composed of negatively charged lipopolysaccharides, which resist damage by EGCG (Reygaert, 2014). EGCG inhibits specific reductases (FabG, FabI) in bacterial type II fatty acid synthesis (Zhang & Rock, 2004). Studies have shown that EGCG inhibits the enzyme dihydrofolate reductase, thereby hindering folate synthesis in bacteria (Navarro-Martínez et al., 2005). Recently, the majority of the antibacterial effects of EGCG, better characterized as antioxidant properties, on a target organism have been linked to EGCG-derived H_2O_2 and oxidative degradation products, and the basis of such findings is challenged by this study.

One study reported that *in vitro* EGCG-derived H_2O_2 inhibited *Pseudomonas aeruginosa* (Liu et al., 2013). Damage to *E. coli* O157:H7 cell walls after EGCG treatment was shown to be due to H_2O_2 synthesis (Cui et al., 2012). In spite of these observations, the mechanism of EGCG-induced bactericidal action remains unclear. Therefore, deciphering the mechanism by which EGCG

* Corresponding authors at: Key Laboratory of Tea Science of Ministry of Education, Hunan Agricultural University, Changsha, Hunan 410128, China (Y.-S. Gong), National Research Center of Engineering Technology for Utilization of Functional Ingredients from Botanicals, Hunan Agricultural University, Changsha, Hunan 410128, China (Z.-H. Liu).

E-mail addresses: gongyushun@hunau.net (Y.-S. Gong), larkin-liu@163.com (Z.-H. Liu).

http://dx.doi.org/10.1016/j.foodchem.2016.08.098
0308-8146/

exerts its antibacterial effects has value, especially for the health and food industry.

The present study used *E. coli* OP50 as a model bacterium to explore the effects of EGCG. The results revealed that an increase in endogenous reactive oxygen species (ROS) production resulted in *E. coli* inhibition, thereby supporting a bactericidal effect rather than a bacteriostatic effect of EGCG. However, the addition of antioxidant *N*-acetylcysteine (NAC) and treatment under anoxic conditions reduced EGCG's bactericidal effects. In addition, in combination with paraquats, EGCG demonstrated a synergistic bactericidal effect, suggesting the use of EGCG as a prooxidant.

2. Materials and methods

2.1. Chemicals and reagents

EGCG (>95% purity) was obtained by hot water extraction of green tea (*Camellia sinensis*) leaves according to a protocol described previously with modification (Isbrucker, Bausch, Edwards, & Wolz, 2006). For the separation of catechin fraction from the initial hot water extract, ethylacetate was added. By subjecting the catechin fraction in ethanol/water to macroreticular resin separation followed by crystallization, EGCG was obtained. The EGCG so obtained was identified by HPLC and stored as a solution at −20 °C. All of the other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA), unless otherwise stated.

For all experiments, stock solutions were freshly prepared in distilled water, unless otherwise stated, and sterilized by filtration through 0.2-μm membranes before administration.

2.2. Bacterial strains and cultures

The *E. coli* OP50 strain was grown overnight in liquid Luria Bertani (LB) medium at 37 °C in a rotary shaker (180 rpm). The LB medium contained (per liter) 10 g of tryptone (OXOID, UK), 5 g of yeast extract (OXOID), and 5 g of NaCl (Sinopharm Chemical Reagent Co., Shanghai, China). Overnight *E. coli* was diluted (1:100) in LB (with and without EGCG) at pH = 7.0 unless otherwise stated. Cell density was monitored at different time intervals by measuring OD600 over a 24-h period in liquid LB, and colony forming unit (CFU) counts after 18 h in solid LB also indicated bacterial growth. To achieve a stage of oxygen-depleted growth medium, LB was purged with nitrogen for 30 min and the experiment was carried out immediately (Matthijssens, Back, Braeckman, & Vanfleteren, 2008).

Bacteria were harvested after 7 h (stationary phase) in both control or treatment settings by centrifugation at 5000 rpm for 10 min, resuspended in M9 salts and normalized to OD600 = 0.1. After appropriate dilutions, 3 μL of each sample was added to LB agar plates followed by incubation at 37 °C for 18 h. The number of colonies was recorded for each growth condition.

Bacterial cells were harvested by centrifugation at 5000 rpm for 10 min. Pellets were resuspended in M9 and centrifugation was repeated. The final pellet was freshly used or stored at −80 °C until further analysis.

2.3. Stability of EGCG in LB medium

To assess the stability of EGCG under various conditions, EGCG was added to the LB medium at different concentrations and incubated at 37 °C for 0–12 h. The culture samples were collected at different time intervals and sterilized by filtration through 0.2-μm membranes before detection by HPLC.

2.4. H_2O_2 determination in LB medium

H_2O_2 concentrations in the LB medium were measured spectrophotometrically using commercially available kits (Sangon, Shanghai, China), according to the manufacturer's instructions, and compared against an H_2O_2 standard curve (Sangon).

2.5. Determination of intracellular ROS

For the visualization of total intracellular ROS, dichlorofluorescin diacetate was used (Beyotime Institute of Biotechnology, China; Ex 488 nm/Em 525 nm). The intracellular superoxide was assayed using the fluorescence probe dihydroethidium (Beyotime Institute of Biotechnology; Ex 510 nm/Em 615 nm).

2.6. Scanning electron microscopic analysis

Before scanning electron microscopic analysis, bacterial samples were prepared by treating with EGCG and incubating at 37 °C for 18 h. Bacterial cells were then harvested and washed three times by centrifugation (5000 rpm; 10 min) and suspended in M9. Subsequently, the bacterial cells were fixed in a glutaraldehyde buffer (2.5%; pH = 6.8) and treated as described previously (Bury, Jelen, & Kalab, 2001). Briefly, after the filtration step, the bacterial samples were treated with polylysine hydrobromide. After dehydration and critical point drying, the bacteria were sputter-coated with gold and examined under a scanning electron microscope (JSM-6480LV; JEOL, Japan).

2.7. Superoxide dismutase and catalase activity

Superoxide dismutase (SOD) and catalase (CAT) activity was measured spectrophotometrically using commercially available kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions. The protein contents were determined using a commercially available kit (BCA; Auragene, China).

2.8. Quantitative RT-PCR

Total RNA was isolated using Trizol (Invitrogen, USA), and cDNA was synthesized using a cDNA synthesis kit (Takara, Dalian, China). qRT-PCR was carried out using SYBR Premix Ex Taq (Takara, Japan) and Rotor Gene Q (Qiagen, Germany). The expression of *ssrA* gene was used as an endogenous control to normalize the amount of mRNA obtained from a target gene. Samples were run in triplicate, and the primers used are as shown in Table S1.

2.9. Statistical analysis

Data are expressed as means ± standard deviation. All data set comparisons were performed by applying Student *t*-test and one-way analysis of variance. Statistical analyses were performed using SPSS 18.0 (demo version; Armonk, NY) and Origin 8.0 (demo version; Northampton, MA). Significance was established at values of $P < 0.05$.

3. Results

3.1. EGCG showed bactericidal effects in a dose-dependent manner

Upon exposing *E. coli* OP50 in both liquid and solid LB media to increasing concentrations of EGCG (100–1000 μM), a dose-dependent inhibition of *E. coli* proliferation was observed (Fig. 1A, B). The inhibition rates after 18 h of EGCG treatment in solid