JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Enrichment of Yeast Thioredoxin by Green Tea Extract through Activation of Yap1 Transcription Factor in *Saccharomyces cerevisiae*

YOSHIFUMI TAKATSUME, KAZUHIRO MAETA, SHINGO IZAWA, AND YOSHIHARU INOUE*

Laboratory of Molecular Microbiology, Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Uji, Kyoto 611-0011, Japan

Thioredoxin (TRX) is an important antioxidant present in all types of organisms. Besides its role as an antioxidant, TRX protects the gastric mucosa due to its antiinflammatory effect. In addition, TRX decreases allergenicity; therefore, the oral administration of TRX is of considerable interest with respect to its clinical use as well as the development of functional foods containing TRX. We have attempted to enrich the cellular TRX content in *Saccharomyces cerevisiae*, and found that green tea extract (Sunphenon), which is rich in catechins (polyphenols), activates the Yap1 transcription factor, leading to the induction of *TRX2*, a target of Yap1. Production of yeast TRX was monitored by both a *TRX2-lacZ* reporter expression assay and Western blotting using an anti-yeast TRX antibody. Maximal production of TRX was achieved in a medium containing 0.1% green tea extract at pH 7.6. We discuss the underlying mechanism by which green tea extract activates Yap1.

KEYWORDS: Thioredoxin; green tea extract; epigallocatechin gallate; Yap1; yeast; antioxidant; polyphenol; catechin

INTRODUCTION

The budding yeast *Saccharomyces cerevisiae*, also known as baker's yeast, plays an important role in food industries, such as alcohol fermentation and bakery. In addition to its important roles as a food microorganism, *S. cerevisiae* cells are also used as a source of medicine. For example, glutathione (GSH), one of the antioxidants in all types of organisms (*1*), is extracted from yeast cells in an industrial scale. GSH is clinically used as an adjunct to liver function impairment therapy (*2*, *3*). Besides GSH, thioredoxin (TRX) is also a ubiquitous antioxidant. TRX is a low-molecular-weight protein-disulfide oxidoreductase (*4*, *5*). In addition to its role as an antioxidant, TRX protects the gastric mucosa due to its antiinflammatory effect (*6*). Furthermore, TRX reduces disulfide bonds of proteins, resulting in an increase in digestibility, leading to the decrease in allergenicity (*7*). Therefore, oral TRX administration is of considerable interest with respect to not only the clinical use of TRX but also the development of functional foods using TRX.

S. cerevisiae has three isoforms of *TRX* genes (*TRX1*, *TRX2*, and *TRX3*) (*8*, *9*). Of these, *TRX2* expression is induced by several chemicals that cause oxidative stress, such as H_2O_2, *tert*-butyl hydroperoxide, diamide and methyl viologen (paraquat) (*10*). *TRX2* expression under oxidative stress conditions is regulated by Yap1 and Skn7 transcription factors (*10*, *11*). To enrich TRX in yeast cells, several approaches would be applicable, e.g., *TRX* and *YAP1* genes overexpression using the recombinant DNA techniques and the breeding of yeast mutants that gain the constitutive-up function of Yap1 and the constitutive-up expression of *TRX* genes. On the other hand, improvements in yeast culture conditions are promising in terms of overcoming the cost and criticisms regarding genetically modified food. However, none of the chemicals thus far proven to induce *TRX2* expression have been approved for use as food additives.

Tea polyphenols, such as catechin, epicatechin, epigallocatechin, and epigallocatechin gallate, are widely accepted as antioxidative food additives. Voluminous studies have demonstrated that tea polyphenols scavenge many kinds of radical and oxidant in vitro (for review, see *12*−*14*). For example, green tea extract as well as individual polyphenols inhibit the induction of pro-oxidant enzymes such as inducible nitric oxide synthase in cultured macrophages (*15*−*18*). These results indicate that tea polyphenols have reactive oxygen species (ROS)-scavenging activity. On the other hand, catechin and its derivatives were reported to have bactericidal activity, the mechanism of which is yet unclarified. We have previously reported that epigallocatechin gallate gives rise to adverse effects on the protection of *Escherichia coli* cells against oxidative damage caused by H_2O_2 and copper ions (*19*), thus on the basis of this we assumed that epigallocatechin gallate has pro-oxidant activity under certain conditions. Recently, Arakawa et al. (*20*) have reported that epigallocatechin gallate induces the production of H_2O_2

* To whom correspondence should be addressed: Phone: +81 774-38-3773, Fax: +81 774-33-3004, E-mail: inoue@food2.food.kyoto-u.ac.jp, or y_inoue@kais.kyoto-u.ac.jp.

10.1021/jf048818h CCC: $30.25
Published on Web 12/17/2004

under physiological pH, which is the major mechanism by which epigallocatechin gallate exerts its bactericidal activity. In addition, Nakagawa et al. (*21*) also recently reported that epigallocatechin gallate induces H_2O_2 generation, which is the primary mechanism by which epigallocatechin gallate induces Jurkat cell apoptosis. These recent reports support our previous assumption that tea polyphenols may cause oxidative stress. Concomitantly, our previous finding together with recent publications led us to explore the possibility of tea polyphenols functioning as an inducer of oxidative stress response in yeast, leading to TRX production.

In this study, we present evidence that demonstrates that Yap1 is activated by green tea extract and individual polyphenols, and thereby induces *TRX2* expression. We establish the conditions for *TRX* induction and discuss the effect of tea polyphenols on the cellular response in yeast.

MATERIALS AND METHODS

Strains and Media. *S. cerevisiae* yeast strains used in this study have the YPH250 background (*MAT***a** *trp1-*Δ*1 his3-*Δ*200 leu2-*Δ*1 lys2-801 ade2-101 ura3-52*). The construction of *yap1*Δ::*HIS3* and *skn7*Δ::*TRP1* in this genetic background was described previously (*22*). The nutrient media used were the YPD (yeast extract-peptone-dextrose) medium (2% glucose, 1% yeast extract, 2% peptone; pH 5.5) and H (*Hansenula*) medium (1% glucose, 0.2% yeast extract, 0.5% peptone, 0.03% K_2HPO_4, 0.03% KH_2PO_4, 0.01% $MgCl_2$; pH 5.5) (*23*). The minimal medium used was the SD (synthetic dextrose) medium (2% glucose, 0.67% yeast nitrogen base without amino acids; pH 5.5) with appropriate amino acids and bases.

Spot Assay. Wild-type, *yap1*Δ, *skn7*Δ and *yap1*Δ*skn7*Δ cells were cultured in the YPD medium until $A_{610} = 0.1$ and diluted serially with sterilized 0.85% NaCl solution. Each cell suspension (5 μl) was spotted onto SD agar plates supplemented with L-Trp, L-His, L-Leu, L-Lys, adenine, and uracil (40 μg/mL each) containing various concentrations of green tea extract. Cells were cultured at 28 °C for 3 days.

GFP-Tagged Yap1. The nucleocytoplasmic localization of Yap1 was monitored using green fluorescent protein (GFP)-tagged Yap1. The details of the construction of GFP−Yap1 were described previously (*24*). The *yap1*Δ cells carrying GFP−Yap1 were cultured in the SD medium until $A_{610} = 0.5$, and then catechin, epigallocatechin gallate, and green tea extract were added. After 20 min, GFP−Yap1 localization was observed using fluorescence microscopy (BX51, Olympus).

Reporter Genes. *TRX2-lacZ*, *CTT1-lacZ*, and *GPX2-lacZ* were described previously (*10*, *22*, *25*). The artificial Yap1-dependent *lacZ* reporter gene containing three SV40 AP-1 sites and the TATA-box of the *CYC1* promoter (*10*) was subcloned into pRS415. The resultant plasmid, which was designated AP-1-*CYC1-lacZ*, was also used to monitor the Yap1 activity. β-Galactosidase and catalase assays were described previously (*26*). One unit of activity was defined as the amount of enzyme that increases A_{420} by 1000 per min at 30 °C. Protein concentration was determined by the method of Bradford (*27*).

Induction of TRX. Cells carrying the reporter gene were cultured in 50 mL of the SD medium in 200 mL flasks until $A_{610} = 1.0$. Cells were collected by centrifugation and suspended in 50 mL of the medium to be examined, whose pH was adjusted to 7.6 with 2 N NaOH, and then green tea extract (0.1%) was added. The pH of the culture was rechecked to adjust it to 7.6 after the addition of green tea extract. Cells were incubated at 28 °C for 90 min, collected by centrifugation, washed three times with 0.85% NaCl solution, and suspended in 10 mM potassium phosphate buffer (pH 7.0). Cells were disrupted with glass beads, and cell homogenates were centrifuged at 14000 rpm for 10 min at 4 °C. The resultant supernatants (cell extracts) were used for enzyme assay and/or TRX Western blot analysis.

Western Blot Analysis. Cell extracts were prepared as described above, and the resultant cell extracts were subjected to SDS−PAGE. The separated proteins were transferred to a PVDF membrane (Millipore), and TRX was detected using an anti-yeast Trx2 antibody raised in rabbits as described previously (*28*).

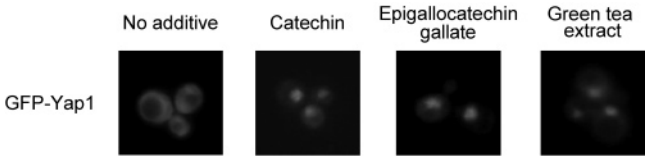

Figure 1. Effect of green tea extract and catechins on the localization of GFP−Yap1. *yap1*Δ cells carrying GFP−Yap1 were cultured in the SD minimal medium until $A_{610} = 0.5$, and then 1% catechin, 0.25% epigallocatechin gallate, and 1% green tea extract were added. After 20 min, GFP−Yap1 localization was monitored by fluorescence microscopy.

Chemicals. Catechin and epigallocatechin gallate were purchased from Wako Pure Chemicals, Osaka, Japan. We used Sunphenon, which was obtained from Taiyo Kagaku, Mie, Japan, as green tea extract. The composition of tea polyphenols in Sunphenon was described previously (*29*).

RESULTS

Green Tea Polyphenols Induce Nuclear Localization of Yap1. Taking into consideration our previous observation (*19*) and recent reports by Arakawa et al. (*20*) and Nakagawa et al. (*21*), catechins seem to cause oxidative stress in bacterial cells and cultured mammalian cells under certain conditions. To determine whether this is also the case in yeast cells, we monitored the intracellular localization of Yap1 in *S. cerevisiae* cells. Yap1 is a redox-sensitive transcription factor, which is predominantly localized in the cytoplasm, whereas it is concentrated in the nucleus under oxidative stress conditions (*24*, *30*). We, therefore, supposed that changes in the nucleocytoplasmic localization of Yap1 are a good indicator of cellular redox potential and/or the degree of oxidative stress conditions (*31*). As shown in **Figure 1**, the GFP-tagged Yap1 was concentrated in the nucleus of cells treated with green tea extract and individual polyphenols, such as catechin and epigallocatechin gallate, suggesting that green tea extract as well as green tea polyphenols cause oxidative stress in yeast.

Mutants Defective in Oxidative Stress-Responsive Transcription Factors Exhibit Sensitivity to Green Tea Extract. Because Yap1 is the critical transcription factor for oxidative stress response in yeast, a *yap1*Δ mutant shows hypersensitivity to H_2O_2 (*32*). In addition to Yap1, the Skn7 transcription factor also plays a crucial role in the transcriptional regulation of many antioxidant genes (*11*, *22*, *33*); therefore, an *skn7*Δ mutant exhibits an increased sensitivity to H_2O_2 (*34*). Because we found that green tea polyphenols activated Yap1 (**Figure 1**), green tea extract likely causes oxidative stress in yeast. If this is the case, mutants defective in these transcription factors are expected to show increased susceptibility to green tea extract due to an impairment of the induction of antioxidant genes. As expected, the *yap1*Δ and *skn7*Δ mutants were sensitive to green tea extract in a dose-dependent manner, and the sensitivity was exacerbated by the simultaneous disruption of *YAP1* and *SKN7* (**Figure 2**). These results strengthen our assumption that green tea extract provokes oxidative stress in yeast.

Green Tea Extract Induces Expression of Yap1 Target Genes. Our data strongly suggest that Yap1 is activated by green tea extract. Several antioxidant genes thus far have been identified to be the target of Yap1. To verify the effect of green tea extract on Yap1 activation, we investigated the expression of some Yap1 target genes (AP-1-*CYC1-lacZ*, *GPX2-lacZ*, and *CTT1-lacZ*) following the treatment with green tea extract under the conditions for *TRX2* expression that we defined, which will be described later in detail.

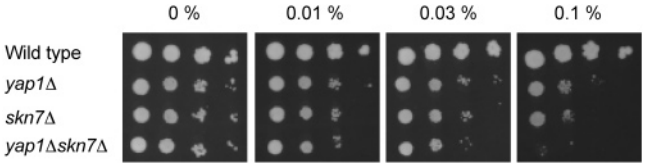

Figure 2. Effect of green tea extract on growth of *yap1*Δ and *skn7*Δ cells. Cells were cultured in the YPD medium until $A_{610} = 0.1$ and diluted serially (1:10). Each cell suspension (5 μL) was spotted on SD agar plates containing various concentrations of green tea extract as indicated in the figure. Cells were cultured at 28 °C for 3 days.

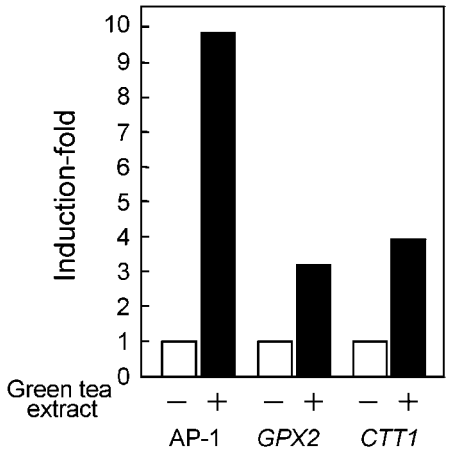

Figure 3. Effect of green tea extract on induction of some Yap1 target genes. Wild-type cells carrying each reporter gene (AP-1, AP-1-*CYC1-lacZ*; *GPX2*, *GPX2-lacZ*; or *CTT1*, *CTT1-lacZ*) were cultured in the SD medium until $A_{610} = 1.0$, collected by centrifugation, and suspended in the H medium at pH 7.6. Cells were cultured for another 90 min in the presence of 0.1% green tea extract, and β-galactosidase activity of each cell extract was determined. Graph indicates the induction-fold of each reporter gene relative to β-galactosidase activity in each untreated cell culture.

Yap1 binds to a specific DNA sequence termed YRE (Yap1 response element, 5′-TTA(C/G)TAA-3′) (*35*). The AP-1-*CYC1-lacZ* reporter gene contains three SV40 AP-1 sites followed by the upstream activating sequence (UAS)-less *CYC1* TATA element and *lacZ* gene (*10*). The nucleotide sequence of the SV40 AP-1 site (5′-TTAGTCA-3′) is close to that of YRE, and the oxidative stress response of this reporter gene is mediated by Yap1 (*10*). Previously, we reported that the expression of *GPX2*, which codes for a phospholipid hydroperoxide glutathione peroxidase homologue in yeast, was mediated by Yap1 and Skn7 (*22*, *36*). The *GPX2* promoter contains both YRE and *cis*-acting element for Skn7 (5′-GGCCGGC-3′, oxidative stress-dependent Skn7 response element, OSRE) (*22*). On the other hand, although the *CTT1* promoter does not contain YRE, interestingly, the oxidative stress-induced expression of *CTT1*, which codes for the cytosolic catalase, is mediated by Yap1 (*37*). As shown in **Figure 3**, the expression of these Yap1-dependent reporter genes was induced by green tea extract. We also confirmed that the catalase activity increased following green tea extract treatment (data not shown).

Green Tea Extract Induces TRX Production. One of the aims of this study is to enrich the TRX content in yeast cells. We have demonstrated that green tea extract activates the expression of Yap1 target genes, and *TRX2* is one of the targets of this transcription factor (*10*); hence, we determined the culture conditions for *TRX2* expression using the plasmid-borne *TRX2-lacZ* reporter gene.

First, we added green tea extract to the yeast culture in the SD medium directly, because we found that the *yap1*Δ and *skn7*Δ mutants exhibited susceptibility to green tea extract in the SD medium (**Figure 2**). β-Galactosidase activity driven by *TRX2-lacZ* increased following the addition of green tea extract to the culture in which yeast cells were growing logarithmically, although the induction-fold was lower (approximately 1.5-fold) than that we expected.

Next, we examined the other two media (YPD medium and H medium) for the inducibility of *TRX2-lacZ*. The YPD medium contains a relatively higher concentration of nitrogen source than the SD and H media. We once used the H medium in the analysis of the oxidative stress response of the yeast *Hansenula mrakii* (*38*, *39*). To avoid the loss of the plasmid-borne *TRX2-lacZ* reporter gene during repeated segregations of cells in such nutrient media, cells were initially cultured in the SD minimal medium until the log phase, and then transferred to the media to be analyzed. Since the addition of green tea extract slightly decreased the pH of the medium, we surveyed the optimal pH of the medium for *TRX2* induction from 5.5, which is the standard pH for yeast media, to 8.0. Interestingly, we found that the expression of *TRX2-lacZ* was efficiently induced by green tea extract under weak alkaline conditions in the SD and H media (**Figure 4A**), although the induction-fold was almost the same (approximately 1.5-fold) ranging from pH 5.5 to pH 6.8 (data not shown). The maximal induction of *TRX2-lacZ* was attained in the H medium at pH 7.6 (**Figure 4A,B**). To verify whether TRX actually increased in cells treated with green tea extract, Western blot analysis was carried out. As a result, the amount of TRX increased in cells following green tea extract treatment depending on the pH of the medium (**Figure 4A**). This result also indicates that the evaluation of *TRX2* expression using this *TRX2-lacZ* reporter gene is valid. Because the antibody that was raised in rabbits against Trx2 produced in *E. coli* cannot distinguish Trx1 and Trx2 (*28*), our data indicates the total amount of Trx1 and Trx2 in yeast cells.

The H medium contains phosphate ions. We therefore determined whether phosphate concentrations affect the induction of *TRX2-lacZ* by green tea extract. As shown in **Figure 4C**, phosphate ions slightly increased the induction-fold up to 10 mM, although phosphate ions at a much higher concentration (100 mM) gave no effect on *TRX2*. induction We also determined the optimal concentration of green tea extract for *TRX2* induction, and 0.1% (w/v) was found to be the minimal concentration to yield the maximal induction. Green tea extract at higher concentrations (0.2−0.5%) did not further enhance *TRX2* induction (data not shown). Next, we observed the time course for the induction of *TRX2-lacZ* expression, and found that it reached the maximal level 60 min after the addition of green tea extract (**Figure 4D**).

Finally, we examined whether TRX expression can be induced by green tea extract without changing the medium. We cultured yeast cells in the H medium until the log phase at pH 5.5, because cell growth was poor at pH 7.6. When A_{610} reached 1.0, the pH of the culture was adjusted to 7.6, and then 0.1% green tea extract was added. The cellular TRX content was determined by Western blotting. As shown in **Figure 5**, the same tendency in terms of TRX induction was obtained as that observed under the conditions that we defined using the *TRX2-lacZ* reporter gene.

Collectively, our data indicate that green tea extract and individual polyphenols activate Yap1, enabling this transcription factor to induce the expression of its target genes, *TRX2* being among the targets.

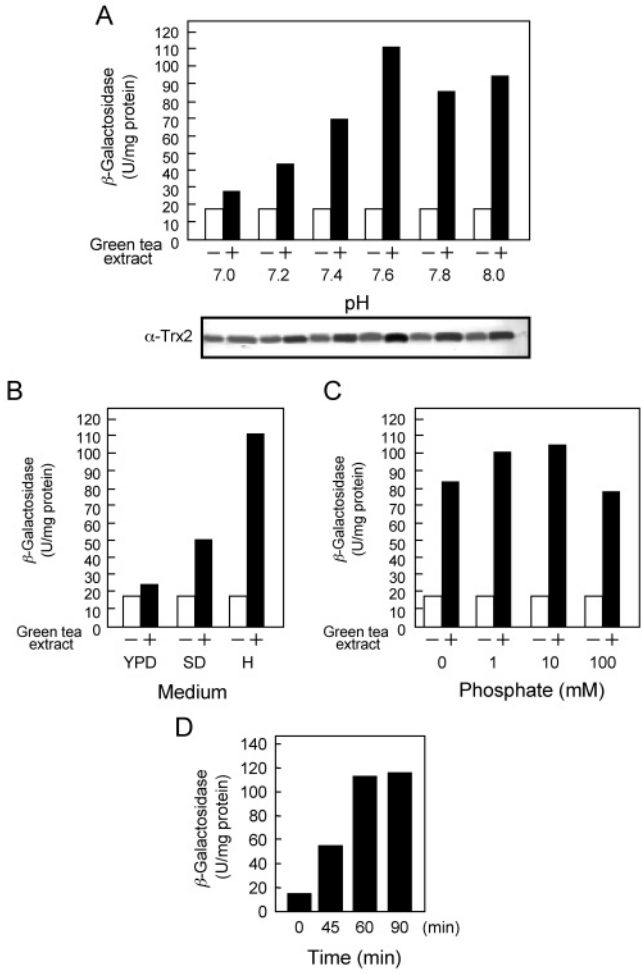

Figure 4. Determination of optimal conditions for *TRX2* expression. (A) Effect of medium pH: Wild-type cells carrying the *TRX2-lacZ* reporter gene were cultured in the SD medium until $A_{610} = 1.0$. Cells were collected by centrifugation, and suspended in the H medium at various pHs. After the addition of 0.1% green tea extract, cells were cultured for 90 min and β-galactosidase activity assay and TRX Western blot analysis were carried out. The samples (40 μg of protein) loaded onto each lane of Western blot analysis (lower picture) correspond to those of the upper graph. (B) Effect of medium composition: Cells were cultured as described above, and suspended in the YPD, SD, or H medium. The pH of each medium was adjusted to 7.6. After 90-min incubation in the presence of 0.1% green tea extract, β-galactosidase activity was measured. (C) Effect of phosphate concentration: Cells were cultured as described above, and suspended in the H medium (pH 7.6) with phosphate ions (as sodium phosphate) at various concentrations. After a 90-min incubation in the presence of 0.1% green tea extract, β-galactosidase activity was measured. (D) Time course: Wild-type cells carrying the *TRX2-lacZ* reporter gene were cultured in the SD medium until $A_{610} = 1.0$. Cells were collected by centrifugation, suspended in the H medium at pH 7.6, and collected at the prescribed time to measure β-galactosidase activity.

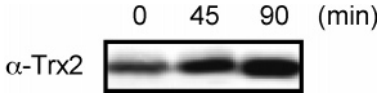

Figure 5. TRX induction in the H medium. Wild-type cells were cultured in the H medium at pH 5.5 until A_{610} reached 1.0. The pH of the culture was adjusted to 7.6 with 2 N NaOH, and 0.1% green tea extract was added. Cells were collected periodically, and TRX content was determined by Western blot analysis using the anti-Trx2 antibody.

DISCUSSION

Polyphenols are widely accepted as antioxidants. Catechin, epigallocatechin, and epigallocatechin gallate are the major polyphenols present in green tea. These tea polyphenols possess ROS-scavenging activity in vitro (for review, see *12*−*14*). Similar to other antioxidants, tea polyphenols also inhibit the activation of redox-sensitive transcription factors, such as NF-κB and AP-1, in cultured mammalian cells through their inhibitory effect on protein kinases that are involved in the activation of such transcription factors; however, the kinase-inhibitory mechanism seems not to be due to their ROS-scavenging activity (*40*). In contrast, we have demonstrated in this study that green tea extract activates the redox-sensitive transcription factor Yap1 in yeast. Our data on Yap1 nuclear localization (**Figure 1**) and the increased susceptibility of the *yap1*Δ and *skn7*Δ mutants to green tea extract (**Figure 2**) led us to examine whether green tea extract causes oxidative stress in yeast. As far as we tested, several Yap1 target genes were found to be induced by green tea extract. The maximal induction of *TRX2* was attained following the treatment of yeast cells with 0.1% green tea extract in the H medium at pH 7.6. The pH dependency of *TRX2* induction is a characteristic aspect. Recently, Arakawa et al. (*20*) reported that tea extract generated H_2O_2 at physiological pH, and approximately 100 μM H_2O_2 was generated by epigallocatechin gallate in a solution containing 100 mM phosphate buffer at a pH >8.0. Under our conditions, phosphate concentrations did not significantly affect *TRX2* induction, although pH was one of the critical factors that influence TRX production. They also reported that almost the same amount of H_2O_2 was produced when the pH ranged from 8 to 10; nevertheless, in the case of *TRX2* induction in yeast, the optimal pH was 7.6 and at a higher pH productivity slightly decreased. Nakagawa et al. (*21*) also reported that epigallocatechin gallate induced the production of H_2O_2 in 100 mM sodium phosphate buffer at pH 7.8, although the H_2O_2 production was small at pH 6.8 and no production was observed at pH 5.8, regardless of the presence of phosphate ions. Taken together, green tea extract may cause oxidative stress in yeast cells, leading to the activation of Yap1.

Alternatively, although Yap1 is a redox-sensitive transcription factor, it is also activated by some drugs, such as benomyl (anti-microtubule drug) and ethylmethane sulfonate (DNA alkylating reagent), resulting in the induction of the expression of some Yap1 target genes such as *FLR1* (*41*). However, both drugs are not classified as the chemicals provoking oxidative stress. On the other hand, both *GSH1* and *GSH2*, coding for γ-glutamyl-cysteine synthetase and glutathione synthetase, respectively, which are the enzymes responsible for GSH biosynthesis, are among the Yap1 targets (*42*, *43*). GSH functions as an antioxidant by supplying electrons to the glutathione peroxidase reaction. In addition, GSH also functions as an antitoxic factor (detoxicant) together with glutathione *S*-transferase (GST), which catalyzes GSH conjugation to electrophiles (xenobiotics) (*44*, *45*). GST is involved in the detoxification enzymes in the phase II reaction, the gene expression of which is mediated via a *cis*-acting element referred to as the antioxidant response element (ARE) (*46*−*49*). For example, *tert*-butyl hydroquinone, an approved food additive possessing an antioxidant activity in vitro, induces the phase II reaction in vivo when taken up by mammalian cells (*50*). Lee et al. (*51*) reported that the polyphenolic fractions of green tea activate phase II enzymes in rat liver. Although we have not examined whether individual polyphenols in green tea extract are taken up by yeast cells, Yap1 activation by catechins may be a phase II-like response of yeast cells.

Another aim of this study was to determine the optimal condition for TRX production in yeast. Here we demonstrated that TRX content was enriched in yeast cells treated with green tea extract. Green tea is a traditional and common beverage in Japan, and its extract is approved for use as a food additive. We have confirmed that catechin and epigallocatechin gallate induced *TRX2* expression (data not shown); nevertheless, green tea extract would be more suitable for TRX production in the industrial scale if we considered the cost performance. To our knowledge, this is the first study demonstrating that yeast TRX production is induced by an approved food additive. Taken together, our method of enriching TRX in yeast cells using green tea extract is promising for food industries that supply TRX-enriched yeast cells as the material for developing TRX-related functional foods.

ABBREVIATIONS USED

GSH, glutathione; TRX, thioredoxin; ROS, reactive oxygen species; GST, glutathione *S*-transferase; YRE, Yap1 response element

ACKNOWLEDGMENT

We thank Dr. S. Kuge for providing plasmids of GFP-Yap1 and *TRX2-lacZ*, and Dr. C. Schüller for the *CTT1-lacZ* plasmid. We are grateful to Dr. J. Yodoi for valuable discussions. Green tea extract (Sunphenon) was donated by Taiyo Kagaku Co., Ltd., Mie, Japan.

LITERATURE CITED

(1) Meister, A. Glutathione-ascorbic acid antioxidant system in animals. *J. Biol. Chem.* **1994**, *269*, 9397−9400.

(2) Vina, J.; Perez, C.; Furukawa, T.; Palacin, M.; Vina, J. R. Effect of oral glutathione on hepatic glutathione levels in rats and mice. *Br. J. Nutr.* **1989**, *62*, 683−691.

(3) Vendemiale, G.; Grattagliano, I.; Altomare, E. An update on the role of free radicals and antioxidant defense in human disease. *Int. J. Clin. Lab. Res.* **1999**, *29*, 49−55.

(4) Holmgren, A. Thioredoxin. *Annu. Rev. Biochem.* **1985**, *54*, 237−271.

(5) Holmgren, A. Thioredoxin and glutaredoxin systems. *J. Biol. Chem.* **1989**, *264*, 13963−13966.

(6) Dekigai, H.; Nakamura, H.; Bai, J.; Tanito, M.; Masutani, H.; Hirota, K.; Matsui, H.; Murakami, M.; Yodoi, J. Geranylgeranylacetone promotes induction and secretion of thioredoxin in gastric mucosal cells and peripheral blood lymphocytes. *Free Radical Res.* **2001**, *35*, 23−30.

(7) del Val, G.; Yee, B. C.; Lozano, R. M.; Buchanan, B. B.; Ermel, R. W.; Lee, Y. M.; Frick, O. L. Thioredoxin treatment increases digestibility and lowers allergenicity of milk. *J. Allergy Clin. Immunol.* **1999**, *103*, 690−697.

(8) Muller, E. G. Thioredoxin genes in *Saccharomyces cerevisiae*: map positions of *TRX1* and *TRX2*. *Yeast* **1992**, *8*, 117−120.

(9) Pedrajas, J. R.; Kosmidou, E.; Miranda-Vizuete, A.; Gustafsson, J. A.; Wright, A. P.; Spyrou, G. Identification and functional characterization of a novel mitochondrial thioredoxin system in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **1999**, *274*, 6366−6373.

(10) Kuge, S.; Jones, N. YAP1 dependent activation of *TRX2* is essential for the response of *Saccharomyces cerevisiae* to oxidative stress by hydroperoxides. *EMBO J.* **1994**, *13*, 655−664.

(11) Morgan, B. A.; Banks, G. R.; Toone, W. M.; Raitt, D.; Kuge, S.; Johnston, L. H. The Skn7 response regulator controls gene expression in the oxidative stress response of the budding yeast *Saccharomyces cerevisiae*. *EMBO J.* **1997**, *16*, 1035−1044.

(12) Frei, B.; Higdon, J. V. Antioxidant activity of tea polyphenols in vitro: evidence from animal studies. *J. Nutr.* **2003**, *133*, 3275S−3284S.

(13) Higdon, J. V.; Frei, B. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. *Crit. Rev. Food Sci. Nutr.* **2003**, *43*, 89−143.

(14) Rice-Evans, C. Plant polyphenols: free radical scavengers or chain-breaking antioxidants? *Biochem. Soc. Symp.* **1995**, *61*, 103−116.

(15) Paquay, J. B.; Haenen, G. R.; Stender, G.; Wiseman, S. A.; Tijburg, L. B.; Bast, A. Protection against nitric oxide toxicity by tea. *J. Agric. Food Chem.* **2000**, *48*, 5768−5772.

(16) Sarkar, A.; Bhaduri, A. Black tea is a powerful chemopreventor of reactive oxygen and nitrogen species: comparison with its individual catechin constituents and green tea. *Biochem. Biophys. Res. Commun.* **2001**, *284*, 173−178.

(17) Chan, M.-M.; Fong, D.; Ho, C.-T.; Huang, H.-I. Inhibition of inducible nitric oxide synthase gene expression and enzyme activity by epigallocatechin gallate, a natural product from green tea. *Biochem. Pharmacol.* **1997**, *54*, 1281−1286.

(18) Lin, Y.-L.; Lin, J.-K. (−)-Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulating lipopolysaccharide-induced activity of transcription factor nuclear factor-kappaB. *Mol. Pharmacol.* **1997**, *52*, 465−472.

(19) Inoue, Y.; Trevanichi, S.; Tsujimoto, Y.; Miki, T.; Miyabe, S.; Sugiyama, K.; Izawa, S.; Kimura, A. Evaluation of catechin and its derivative as antioxidant: recovery of growth arrest of *Escherichia coli* under oxidative conditions. *J. Sci. Food Agric.* **1996**, *71*, 297−300.

(20) Arakawa, H.; Maeda, M.; Okubo, S.; Shimamura, T. Role of hydrogen peroxide in bactericidal action of catechin. *Biol. Pharm. Bull.* **2004**, *27*, 277−281.

(21) Nakagawa, H.; Hasumi, K.; Woo, J. T.; Nagai, K.; Wachi, M. Generation of hydrogen peroxide primarily contributes to the induction of Fe(II)-dependent apoptosis in Jurkat cells by (−)-epigallocatechin gallate. *Carcinogenesis* **2004**, Apr 16 [Epub ahead of print].

(22) Tsuzi, D.; Maeta, K.; Takatsume, Y.; Izawa, S.; Inoue, Y. Regulation of the yeast phospholipid hydroperoxide glutathione peroxidase *GPX2* by oxidative stress is mediated by Yap1 and Skn7. *FEBS Lett.* **2004**, *565*, 148−154.

(23) Inoue, Y.; Trevanichi, S.; Fukuda, K.; Izawa, S.; Wakai, Y.; Kimura, A. Roles of esterase and alcohol acetyltransferase on production of isoamyl acetate in *Hansenula mrakii*. *J. Agric. Food Chem.* **1997**, *45*, 644−649.

(24) Kuge, S.; Jones, N.; Nomoto, A. Regulation of yAP-1 nuclear localization in response to oxidative stress. *EMBO J.* **1997**, *16*, 1710−1720.

(25) Wieser, R.; Adam, G.; Wagner, A.; Schüller, C.; Marchler, G.; Ruis, H.; Krawiec, Z.; Bilinski, T. Heat shock factor-independent heat control of transcription of the *CTT1* gene encoding the cytosolic catalase T of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **1991**, *266*, 12406−12411.

(26) Inoue, Y.; Tsujimoto, Y.; Kimura, A. Expression of the glyoxalase I gene of *Saccharomyces cerevisiae* is regulated by high osmolarity glycerol mitogen-activated protein kinase pathway in osmotic stress response. *J. Biol. Chem.* **1998**, *273*, 2977−2983.

(27) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

(28) Izawa, S.; Maeda, K.; Sugiyama, K.; Mano, J.; Inoue, Y.; Kimura, A. Thioredoxin deficiency causes the constitutive activation of Yap1, an AP-1-like transcription factor in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **1999**, *274*, 28459−28465.

(29) Kudo, M.; Naito, Z.; Yokoyama, M.; Asano, G. Effects of quercetin and sunphenon on responses of cancer cells to heat shock damage. *Exp. Mol. Pathol.* **1999**, *66*, 66−75.

(30) Delaunay, A.; Isnard, A. D.; Toledano, M. B• H_2O_2 sensing through oxidation of the Yap1 transcription factor. *EMBO J.* **2000**, *19*, 5157−5166.

(31) Izawa, S.; Inoue, Y. A screening system for antioxidants using thioredoxin-deficient yeast: discovery of thermostable antioxidant activity from *Agaricus blazei* Murill. *Appl. Microbiol. Biotechnol.* **2004**, *64*, 537−542.
(32) Schnell, N.; Krems, B.; Entian, K. D. The *PAR1* (*YAP1*/*SNQ3*) gene of *Saccharomyces cerevisiae*, a c-jun homologue, is involved in oxygen metabolism. *Curr. Genet.* **1992**, *21*, 269−273.
(33) Lee, J.; Godon, C.; Lagniel, G.; Spector, D.; Garin, J.; Labarre, J.; Toledano, M. B. Yap1 and Skn7 control two specialized oxidative stress response regulons in yeast. *J. Biol. Chem.* **1999**, *274*, 16040−16046.
(34) Krems, B.; Charizanis, C.; Entian, K. D. The response regulator-like protein Pos9/Skn7 of *Saccharomyces cerevisiae* is involved in oxidative stress resistance. *Curr. Genet.* **1996**, *29*, 327−334.
(35) Fernandes, L.; Rodrigues-Pousada, C.; Struhl, K. Yap, a novel family of eight bZIP proteins in *Saccharomyces cerevisiae* with distinct biological functions. *Mol. Cell. Biol.* **1997**, *17*, 6982−6993.
(36) Inoue, Y.; Matsuda, T.; Sugiyama, K.; Izawa, S.; Kimura, A. Genetic analysis of glutathione peroxidase in oxidative stress response of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **1999**, *274*, 27002−27009.
(37) Winderickx, J.; de Winde, J. H.; Crauwels, M.; Hino, A.; Hohmann, S.; Van Dijck, P.; Thevelein, J. M. Regulation of genes encoding subunits of the trehalose synthase complex in *Saccharomyces cerevisiae*: novel variations of STRE-mediated transcription control? *Mol. Gen. Genet.* **1996**, *252*, 470−482.
(38) Tran, L.-T.; Inoue, Y.; Kimura, A. Oxidative stress response in yeast: purification and some properties of a membrane-bound glutathione peroxidase from *Hansenula mrakii*. *Biochim. Biophys. Acta* **1993**, *1164*, 166−172.
(39) Inoue, Y.; Tran, L.-T.; Kamakura, M.; Izawa, S.; Miki, T.; Tsujimoto, Y.; Kimura, A. Oxidative stress response in yeast: glutathione peroxidase of *Hansenula mrakii* is bound to the membrane of both mitochondria and cytoplasm. *Biochim. Biophys. Acta* **1995**, *1245*, 325−330.
(40) Yang, C. S.; Maliakal, P.; Meng, X. Inhibition of carcinogenesis by tea. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 25−54.
(41) Nguyen, D. T.; Alarco, A. M.; Raymond, M. Multiple Yap1-binding sites mediate induction of the yeast major facilitator *FLR1* gene in response to drugs, oxidants, and alkylating agents. *J. Biol. Chem.* **2001**, *276*, 1138−1145.
(42) Wu, A. L.; Moye-Rowley, W. S. *GSH1*, which encodes γ-glutamylcysteine synthetase, is a target gene for yAP-1 transcriptional regulation. *Mol. Cell. Biol.* **1994**, *14*, 5832−5839.
(43) Sugiyama, K.; Izawa, S.; Inoue, Y. The Yap1p-dependent induction of glutathione synthesis in heat shock response of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **2000**, *275*, 15535−15540.
(44) Sheweita, S. A.; Tilmisany, A. K. Cancer and phase II drug-metabolizing enzymes. *Curr. Drug Metab.* **2003**, *4*, 45−58.
(45) Hayes, J. D.; Pulford, D. J. The glutathione *S*-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit. Rev. Biochem. Mol. Biol.* **1995**, *30*, 445−600.
(46) Yu, R.; Jiao, J.-J.; Duh, J.-L.; Gudehithlu, K.; Tan, T.-H.; Kong, A.-N. Activation of mitogen-activated protein kinases by green tea polyphenols: potential signaling pathways in the regulation of antioxidant-responsive element-mediated phase II enzyme gene expression. *Carcinogenesis* **1997**, *18*, 451−456.
(47) Chen, C.; Yu, R.; Owuor, E.-D.; Kong, A.-N. Activation of antioxidant-response element (ARE), mitogen-activated protein kinases (MAPKs) and caspases by major green tea polyphenol components during cell survival and death. *Arch. Pharm. Res.* **2000**, *23*, 605−612.
(48) Lin, Y.-L.; Cheng, C.-Y.; Lin, Y.-P.; Lau, Y.-W.; Juan, I.-M.; Lin, J.-K. Hypolipidemic effect of green tea leaves through induction of antioxidant and phase II enzymes including superoxide dismutase, catalase, and glutathione-S-transferase in rats. *J. Agric. Food Chem.* **1998**, *46*, 1893−1899.
(49) Jowsey, I. R.; Jiang, Q.; Itoh, K.; Yamamoto, M.; Hayes, J. D. Expression of the aflatoxin B1−8,9-epoxide-metabolizing murine glutathione *S*-transferase A3 subunit is regulated by the Nrf2 transcription factor through an antioxidant response element. *Mol. Pharmacol.* **2003**, *64*, 1018−1028.
(50) Dhakshinamoorthy, S.; Jaiswal, A. K. c-Maf negatively regulates ARE-mediated detoxifying enzyme genes expression and antioxidant induction. *Oncogene* **2002**, *21*, 5301−5312.
(51) Lee, S.-F.; Liang, Y.-C.; Lin, J.-K. Inhibition of 1,2,4-benzenetriol-generated active oxygen species and induction of phase II enzymes by green tea polyphenol. *Chem. Biol. Interact.* **1995**, *98*, 283−301.

Received for review July 15, 2004. Revised manuscript received October 26, 2004. Accepted November 2, 2004. This study was supported by grants from BRAIN.

JF048818H