Biochemical Characterization of 60S Acidic Ribosomal P Proteins Associated with CK-II from Bamboo Shoots and Potent Inhibitors of Their Phosphorylation in Vitro

Toshiro MAEKAWA,a Seiji KOSUGE,a Sakı SAKAMOTO,a Shinji FUNAYAMA,b Kanki KOMIYAMA,b and Kenzo OHITSUKI,a,c

Laboratory of Genetical Biochemistry, School of Allied Health Sciences, Kitasato University,a 1-15-1 Kitasato, Sagamihara 228-8555, Japan, Department of Bioscience and Biotechnology, Aomori University,b 2-3-1 Kohbata, Aomori 030-0943, Japan, and The Kitasato Institute,c 5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan.

Received February 12, 1999; accepted April 26, 1999

Three effective phosphate acceptors (35, 15 and 13 kDa polypeptides) for casein kinase II (CK-II) in the Superdex CK-II fraction prepared from a 0.5 m NaCl extract of bamboo shoots were selectively purified by glycyrrhizin (GL)-affinity column chromatography (HPLC). These three proteins (p35, p15 and p13) were identified as 60S acidic ribosomal P proteins by determination of their partial N-terminal sequences. CK-II was associated with p35 since the GL-affinity fraction was coprecipitated with an anti-serum against Drosophila CK-IIβ. Moreover, a derivative (oGA) of glycyrrhetinic acid (GA) and several polyphenol-containing anti-oxidative compounds [quercetin, epigallocatechin gallate (EGCG) and two iso-flavones, i.e., 3',4',7-trihydroxyisoflavone (3',4',7-THT) and 8-chloro-3',4',5,7-tetrahydroxyisoflavone (8C-3',4',5,7-THT)] inhibited the CK-II-mediated phosphorylation of 60S acidic ribosomal P proteins in vitro. Quercetin was found to be the most effective compound on CK-II activity since its ID50 was approx. 50 nM. These results suggest that (i) GL-affinity column chromatography is useful for the selective purification of 60S acidic ribosomal P proteins as a heterocomplex associated with CK-II from various cell sources; (ii) natural anti-oxidative compounds with polyphenols, but not GL and GA, act as potent CK-II suppressors; and (iii) CK-II mediates the regulation of the physiological functions of 60S acidic ribosomal P proteins in growing plant cells.

Key words 60S acidic ribosomal P protein; casein kinase II; phosphorylation; CK-II inhibitor; glycyrrhizin; anti-oxidative compound

Glycyrrhizin (GL) is present in large quantities in the roots and rhizomes of licorice, Glycyrrhiza glabra L., and is composed of a molecule of glycyrrhetinic acid (GA) and two molecules of glucuronic acid. Recently, we reported that (i) casein kinase II (CK-II) in the partially purified fraction from mouse liver is selectively purified by GL-affinity column chromatography (HPLC) as a GL-binding protein (gbP)2; (ii) GL at above 20 μM inhibits the CK-II-mediated phosphorylation of cellular functional proteins, such as a nuclear receptor (glucocorticoid receptor), lipotocin 1,2 lipoxigenase 3 (LOX-3)3 and HIV-1 reverse transcriptase,4 in vitro; and (iii) a derivative (oGA) of GA, as well as quercetin, at lower doses (approx. 1 μM) selectively inhibits CK-II activity in vitro.1,5

CK-II is a highly conserved, cAMPγ, guanosine 3',5'-cyclic monophosphate (cGMP)- and Ca2+-independent serine/threonine protein kinase, which has been characterized in numerous mammalian6,7 and plant cells.8,9 In mammalian cells, it has been shown that CK-II plays important roles in the initiation of DNA replication and transcription: it specifically modifies DNA-binding proteins (DNA topoisomerases and SV-40 large T antigen), transcriptional factors [Sp1, serum response factor (SRF) and Ap-1], and some oncogen products (erbAα, Myb and Myc).5,7 In addition, CK-II plays an important physiological role in protein synthesis since it phosphorylates initiation factors (eIFs)9–11 and 60S acidic ribosomal P proteins (P0, P1 and P2).12 A recent report has shown that (i) human ribosomal protein L5 interacts with the β-subunit of CK-II; and (ii) the protein L5 may act as a regulator of the activity or subcellular localization of CK-II.13,14 However, the biological significance of CK-II among the regulatory mechanisms of protein synthesis in plant cells is currently unclear. Therefore, the present study was undertaken to examine the direct physiological interaction between CK-II and ribosomal P proteins in rapidly growing plant cells, such as bamboo shoots, and also to determine the inhibitory effects of GL, GA, oGA and natural polyphenol-containing anti-oxidative compounds [catechin, quercetin, epigallocatechin gallate (EGCG) and four iso-flavones, i.e., genisten (3',5,7-TH), 4',7,8-trihydroxyisoflavone (4',7,8-TH), 3',4',7-trihydroxyisoflavone (3',4',7-TH) and 8-chloro-3',4',5,7-tetrahydroxyisoflavone (8C-3',4', 5,7-TH)] on the CK-II-mediated phosphorylation of ribosomal P proteins in vitro.

Here, we describe (i) the selective purification of three effective phosphate acceptors (p35, p15 and p13) for CK-II from a 0.5 m NaCl crude extract of bamboo shoots by GL-affinity column chromatography, (ii) the identification of these three phosphate acceptors by determination of their amino acid sequences, and (iii) the biochemical characterization of a GA derivative (oGA) and several polyphenol-containing anti-oxidative compounds (catechin, quercetin, EGCG and four iso-flavones) as potent CK-II inhibitors in vitro.

MATERIALS AND METHODS

Chemicals [γ-32P]ATP (3000 Ci/mm mol) was obtained from Amersham Pharmacia Biotech (Buckinghamshire, England); dithiothreitol (DTT), phenylmethylsulfonyl fluoride (PMSF), poly-L-arginine (poly-Arg), heparin, dephosphorylated α-casein (bovine milk), catechin, quercetin and EGCG
from Sigma Chemical (St. Louis, U.S.A.); and polyvinylidene fluoride (PVDF) membrane from Bio-Rad Laboratories (California, U.S.A.). Three isoflavones (4',7,8-THI, 3',4',7-THI and 8C-3',4',5,7-THI) were isolated and purified from the culture filtrates of Streptomyces sp. OHI-1049.\textsuperscript{15,16} Antiserum (rabbit) against Drosophila CK-II\textbeta was kindly supplied by Dr. S. Nakajo (Showa Univ., Tokyo 142–8555, Japan).

**GL-Affinity Column** A GL-affinity column was prepared as originally described by Nakamura \textit{et al.}\textsuperscript{17} using Tresyl-5PW (packing gel for HPLC; Tosoh Mfg. Co., Ltd., Tokyo) and N-(glycyrrhizin)-30-\alpha-lysine, as previously reported.\textsuperscript{18}

**Extraction of CK-II and Its Phosphate Acceptors from Bamboo Shoots** To extract CK-II and its phosphate acceptors from the young tissues of bamboo shoots, the tops (about 120 g; only soft tissues without outer skins) of fresh bamboo shoots were cut into small pieces and homogenized in 360 ml of buffer A [20 mm Tris–HCl (pH 7.6), 2 mm DTT and 1 mm PMSF] containing 0.5 m NaCl. The crude extract was concentrated with 70% saturated ammonium sulfate. The resulting precipitates were dissolved in 50 ml of buffer A containing 0.1 m NaCl. After dialysis against the same buffer, the ammonium sulfate fraction (about 450 mg protein) was used as a crude CK-II extract from bamboo shoots.

**Partial Purification of CK-II and Its Phosphate Acceptors by DEAE-Cellulose Column Chromatography** A crude bamboo shoot extract (about 200 mg protein) was applied to a DEAE-cellulose column (3.5×15 cm) previously equilibrated with buffer A containing 0.15 m NaCl. The charged proteins on the column were eluted step-wise with 0.15 and 1.0 m NaCl. The 1.0 m NaCl fraction (D-II fraction) was used as a partially purified CK-II fraction, since CK-II and its three phosphate acceptors (p35, p15 and p13) were mainly detected in this fraction.

**Assay for CK-II Activity** CK-II activity was measured in the standard reaction mixture (100 \( \mu l \)), which was comprised of 40 mm Tris–HCl (pH 7.6), 2 mm DTT, 3 mm Mn\textsuperscript{2+}, 3 \( \mu g \) of \( \alpha \)-casein (phosphate acceptor), 20 \( \mu m \) \( [\gamma\textsuperscript{32}P] \) ATP (1000 cpm/pmol), and the indicated amount of purified CK-II or recombinant human CK-II (rhCK-II). After incubation for the indicated periods at 30°C in the presence or absence of poly-Arg (5 \( \mu g \), CK-II activator), the \( [\gamma\textsuperscript{32}P] \)-labeled casein on the filter was determined with a liquid scintillation spectrometer, as reported previously.\textsuperscript{4,5}

**Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Autoradiography** After incubation with 20 \( \mu m \) \( [\gamma\textsuperscript{32}P] \) ATP (1000 cpm/pmol) in the presence or absence of poly-Arg, SDS-PAGE followed by autoradiography was performed to detect \( [\gamma\textsuperscript{32}P] \)-labeled polypeptides in the indicated CK-II fractions, as reported previously.\textsuperscript{4,5}

**Determination of Amino Acid Sequences** Polypeptides electrophoretically blotted on a PVDF membrane were detected by staining with amido black 10B, and the bands on the membrane with a clean zone were cut out. Each piece was placed in a cartridge block for the sequencer. The primary structures of targeting polypeptides were determined by a protein sequencer (model PQS-1, Shimadzu Co., Ltd., Kyoto, Japan). To identify the purified polypeptides as functional gene products, their partial amino acid sequences were subjected to a Bio-database.

**RESULTS**

**Selective Purification of CK-II and Its Three Phosphate Acceptors** CK-II and its phosphate acceptors in the DEAE-cellulose fraction (D-II fraction) prepared from a 0.5 m NaCl extract of bamboo shoots were further purified by heparin-affinity column chromatography (HPLC). CK-II activity was detected in the fractions eluted between 0.8 and 1.0 m NaCl (Fig. 1A). Polypeptide analysis of the heparin-affinity fraction by SDS-PAGE (Fig. 1B) and autoradiography (Fig. 1C) detected three major phosphorylated polypeptides (approx. 35, 15 and 13 kDa). These three polypeptides (p35, p15 and p13) in the heparin-affinity fraction were further purified by gel filtration on a Superdex 200 pg HPLC column. The CK-II activity was detected in fractions with molecular sizes between 200 and 250 kDa (Fig. 2A). SDS-PAGE (Fig. 2B) and autoradiography (Fig. 2C) detected three polypeptides in the peak fraction (fraction 12) of CK-II. Figure 2C shows that phosphorylation of these three polypeptides by CK-II is remarkably stimulated by poly-Arg (lane 2), but inhibited by heparin (lane 3) or cold GTP (lane 4). These
characteristics of the CK-II-mediated phosphorylation of these three proteins (p35, p15 and p13) from bamboo shoots are similar to those observed with protein phosphorylation by CK-Is from mammalian cells.5–7 Finally, CK-II and its three phosphate acceptors in the Superdex fraction were purified by GL-affinity column chromatography (HPLC). CK-II activity was detected in the fractions eluted between 0.5 and 0.6 M NaCl (Fig. 3A). SDS-PAGE detected three polypeptides (p35, p15 and p13) in the peak fraction 27 of CK-II (Fig. 3B). These polypeptides were phosphorylated when fraction 27 was directly incubated with 20 μM [γ-32P]ATP (1000 cpm/pmol) in the presence of poly-Arg (lane 3, Fig. 3C). These results show that the CK-II phosphate acceptors (p35, p15 and p13) are copurified with CK-II from a 0.5 M NaCl extract of bamboo shoots.

Identification of the Three CK-II Phosphate Acceptors (p35, p15 and p13) To identify the three CK-II phosphate acceptors purified from bamboo shoots, their amino acid sequences were determined. The 20 N-terminal amino acids [AIKRTKAEKKQAYQKKLQQL] of p35 were 85% identical to the corresponding sequence of 60S acidic ribosomal protein P0 in rice.19 In contrast, the 16 N-terminal amino acids [MKVVAAYLLAVLGNP] of p13 were 90% identical to the corresponding sequence of 60S acidic ribosomal protein P2 in maize.20 In the case of p15, its N-terminal 8 amino acids [GVYTFVYR] were 87.5% identical to P1/P2 type protein (approx. 15 kDa, named P3), which has a highly conserved C terminus of P1 and P2 in maize seedling roots.21 The amino acid sequences of these three ribosomal proteins (p35, p15 and p13) from bamboo shoots were more than 85% identical to 60S acidic ribosomal P proteins (P0, P1 and P2) in mammalian cells.22,23 These results, therefore, suggest that three effective phosphate acceptors (p35, p15 and p13) for CK-II purified from bamboo shoots are classified in the family of 60S acidic ribosomal P proteins.

Association of CK-II with 60S Acidic Ribosomal P Proteins To confirm the presence of protein kinase in the purified 60S acidic ribosomal P proteins (p35, p15 and p13) from bamboo shoots, the GL-affinity fraction (fraction 27, Fig. 3A) was incubated with anti-serum against Drosophila CK-IIβ. As expected, p35 was detected when the immunoprecipitates were directly incubated with 20 μM [γ-32P]ATP (1000 cpm/pmol) in the presence of poly-Arg (lane 3, Fig. 4). Under the experimental conditions, α-casein was also phosphorylated when the immunoprecipitates were incubated in the presence of 20 μM [γ-32P]ATP and poly-Arg (lane 4, Fig.
MW (kDa) 66 45 31 21.5 14.5 6.5

![MW Graph](image)

Fig. 4. Immunoprecipitation of the GL-Affinity Fraction with Anti-serum of Drosophila CK-IIβ and Detection of CK-II-Mediated Phosphorylation of p35

To identify the protein kinase associated with the 60S acidic ribosomal P proteins (p35, p15 and 13) as CK-II, GL-affinity fraction 27 (Fig. 3A) was incubated for 2 h at 4°C with anti-serum of Drosophila CK-IIβ or normal rabbit serum in the presence of protein A-Sepharose. The resulting immunoprecipitates were incubated directly for 30 min at 30°C with [γ-32P]ATP in the presence of poly-Arg. The 32P-labelled polypeptides in the reaction mixtures were detected by SDS-PAGE, followed by autoradiography. Lane 1, GL-affinity fraction 27 with normal rabbit serum; lane 2, GL-affinity fraction 27 precipitated with anti-serum against Drosophila CK-IIβ; lane 3, GL-affinity fraction 27 precipitated with the anti-CK-IIβ serum in the presence of poly-Arg (0.5 μg/ml); lane 4, lane 3 + α-casein (an exogenous phosphate acceptor for CK-II).

4). These results show that (i) a protein kinase associated with the complex of 60S acidic ribosomal P proteins is identified as CK-II; (ii) the β-subunit of CK-II associated with 60S acidic ribosomal P proteins from bamboo shoots has a homology with Drosophila CK-IIβ; and (iii) CK-II is responsible for the specific phosphorylation of these 60S acidic ribosomal P proteins in the presence of a suitable kinase activator, such as poly-Arg, in vitro.

Characterization of Potent CK-II Inhibitors in Vitro

The inhibitory effects of GL, GA, gGA and natural polyphenol-containing anti-oxidative compounds [catechin, quercetin, EGCG and four isoflavones (genistein, 4',7,8,THI, 3',4',7,THI and 8C-3',4',5,7-THI) and 8C-3',4',5,7-THI] (Fig. 5)] on CK-II activity (phosphorylation of 60S acidic ribosomal P proteins) were determined in vitro. In this experiment, the phosphorylation of p35 (P0) by CK-II was slightly more sensitive to quercetin, as compared with sensitivity to the phosphorylation of p15 (P3) and p13 (P2) (Fig. 6B). Quercetin inhibited CK-II activity in a dose-dependent manner (Fig. 6A) and its 50% inhibition (ID50) was found to be approx. 50 nm (Fig. 6B). As shown in Figure 6C, there was a substantial alteration in V̇max for recombinant human CK-II (rhCK-II), from 50.0 to 28.5 pmol/min, when 30 nm quercetin was added to the reaction mixtures. However, no change in Km (approx. 0.125 μg/ml) for the substrate (α-casein) was detected under the given experimental conditions. A similar inhibitory effect of quercetin on CK-II activity was observed when other CK-II substrates, such as Hsp-90, phosphitin and 60S acidic ribosomal P proteins were used instead of α-casein as a phosphate acceptor (data not shown).

Two flavones (quercetin and EGCG) and four isoflavones (genistein, 4',7,8,THI, 3',4',7,THI and 8C-3',4',5,7-THI) with polyphenols inhibited CK-II activity in a dose-dependent manner, as shown for the inhibitory kinetics of quercetin (Fig. 6B). Table 1 shows that (i) quercetin (ID50 = 50 nm) is the most effective inhibitor of CK-II; and (ii) EGCG (ID50 = 0.3 μm) as well as 8C-3',4',5,7-THI (ID50 = 0.1 μm) effectively inhibit CK-II activity in vitro. These results suggest that natural polyphenol-containing anti-oxidative compounds, such as quercetin, EGCG and 8C-3',4',5,7-THI, function as potent CK-II inhibitors of 60S acidic ribosomal P proteins at the cellular level.

At low doses (0.1—1 μm), GL significantly stimulated CK-II activity, but inhibited it at doses above 30 μm. GA completely inhibited CK-II activity [phosphorylation of P0 protein (p35)] at one hundredth the concentration of GL (Fig. 7).

DISCUSSION

CK-II and its effective phosphate acceptors (p35, p15 and p13) were partially purified from a 0.5 M NaCl extract of bamboo shoots, by means of DEAE-cellulose column chromatography, heparin-affinity column chromatography (HPLC), gel filtration on a Superdex 2000 pg HPLC column and GL-affinity column chromatography (HPLC), successively (Figs. 1—3). Determination of the N-terminal amino acid sequences of these three CK-II phosphate acceptors (p35, p15 and p13) revealed that three protein substrates belong to the family of 60S acidic ribosomal P proteins, which are highly conserved among prokaryotic and eukaryotic species.25—27 Indeed, these 60S acidic ribosomal P proteins in partially purified CK-II fractions (Superdex fractions) prepared from spinach, rat liver and mouse brain could be separated by means of GL-affinity column chromatography (data not shown). Therefore, a GL-affinity column is a useful, effective tool to selectively purify the 60S acidic ribosomal P proteins from various cell sources.

The 60S acidic ribosomal P proteins (P0, P1 and P2) are well-characterized in prokaryotes23 as well as eukaryotes,22—24 forming a pentameric complex (P0—P1—P2), which comprises the eukaryotic ribosomal stalk.22 It has been shown that one P1 homodimer and one P2 homodimer are attached to P0 via their NH₂-terminal ends.22 The COOH-terminal 12 amino acids (ESDDDMGFGLFD) of P0, P1, P2 and P3 are almost identical, except for conservative amino acid substitutions, are highly conserved between species,26 and are specifically phosphorylated by CK-II.27 Recently, it has been shown that CK-II mediates the physiological interaction of P2 with an elongation factor eEF-2.28 However, the biological significance of CK-II in the physiological activities of the functional ribosomal P proteins is still unclear.

Our observations that (i) CK-II is copurified with a heterocomplex of three 60S acidic ribosomal P proteins (Figs. 2 and 3); (ii) CK-II effectively phosphorylates these ribosomal P proteins in the presence of a suitable CK-II activator in vitro (Figs. 2—4); and (iii) an anti-serum against Drosophila CK-IIβ can coprecipitate p35 in the GL-affinity fraction 27 after incubation with [γ-32P]ATP and poly-Arg (Fig. 4), suggest that (i) the β-subunit of CK-II associated with 60S acidic ribosomal P proteins from bamboo shoots has a homology with Drosophila CK-IIβ and (ii) CK-II is a protein kinase responsible for the phosphorylation of 60S acidic ribosomal P proteins in vitro. These observations also suggest that the physiological activity of the 60S acidic ribosomal P proteins may be regulated through their specific phosphorylation.
tion by CK-II at the cellular level. This conclusion basically corresponds to the phosphorylation of 60S acidic ribosomal P proteins by endogenous CK-II or exogenous CK-II like kinase, as originally reported by Hasler et al. in mammalian cells.27)

To investigate the inhibitory effects of GL, GA, oGA, and natural polyphenol-containing anti-oxidative compounds [catechin, quercetin, EGCG and four isoflavones (Fig. 5)] on CK-II activity (phosphorylation of 60S acidic ribosomal P proteins) in vitro, the Superdex fraction 12 was incubated with 20 μM [γ-32P]ATP and poly-Arg in the presence of these compounds. GL at lower doses (0.1–1 μM) significantly stimulated CK-II activity, as demonstrated in the phosphorylation of Hsp-90 as well as in Ca2+-binding protein (calreticulin) by CK-II in rat liver.11 In contrast, GL inhibited CK-II activity in a dose-dependent manner at doses above 30 μM (Fig. 5). Similar kinetics were observed with GA (Fig. 5). oGA inhibited CK-II activity in a dose-dependent manner, as previously reported for CK-II from rat liver.14 These results suggest that (i) the GL-binding sites on the 60S acidic ribosomal P proteins are different from their phosphorylation sites for CK-II; and (ii) oGA acts as an effective inhibitor for CK-II in vitro.

Akiyama et al. reported that genistein (4',5,7,THI) is a specific inhibitor of tyrosine-specific protein kinases, such as epidermal growth factor (EGF) receptor (ID50 = approx. 2.7 μM), pp60c-src and pp110b-fes, but not of A-kinase and C-kinase in vitro.29) Using our in vitro CK-II assay system, the inhibitory effects of natural polyphenol-containing anti-oxidative compounds [catechin, quercetin, EGCG and four isoflavones (genistein, 4',7,8-THI, 3',4',7-THI and 8C-3',4',5,7-THI)] were examined. It was found that (i) natural polyphenol-containing anti-oxidative compounds [two flavones (quercetin and EGCG) and three isoflavones (4',7,8-THI, 3',4',7-THI and 8C-3',4',5,7-THI)] inhibit CK-II activity at lower doses (Table 1); (ii) quercetin (ID50 = 50 nm) is the most effective inhibitor of CK-II in vitro (Fig. 5B); (iii) these CK-II inhibitors directly bind to the α-subunit of CK-II and also inhibit its autophosphorylation (data not shown). However, no significant effect of genistein (ID50 = 20 μM) on
Fig. 6. Inhibitory Kinetics of the CK-II-Mediated Phosphorylation of 60S Acidic Ribosomal P Proteins (p35, p15 and p13) by Quercetin in Vitro

[A] To determine the inhibitory kinetics of CK-II activity (phosphorylation of 60S acidic ribosomal P proteins) by quercetin, the purified CK-II fraction (Superdex fraction 12, Fig. 2A) was incubated with 20 μM [γ-32P]ATP and poly-Arg (0.5 μg/ml) in the presence or absence of various concentrations of quercetin. After incubation, the 32P-labelled p35, p15 and p13 in the reaction mixtures were detected by SDS-PAGE followed by autoradiography. [B] The autoradiogram was scanned by a spectrophotometer, and 100% represents the CK-II-mediated phosphorylation of p33, p15 and p13 determined without quercetin. Phosphorylation of p15 (□), p15 (△) and p13 (○). [C] Kinetics of quercetin-induced inhibition of CK-II activity in vitro. Double-reciprocals of plots of inhibition by quercetin against CK-II activity. Recombinant human CK-II (rCK-II, approx. 30 ng) was incubated with 20 μM [γ-32P]ATP and α-casein (phosphate acceptor) in the presence or absence of 30 μM quercetin. The 32P-labelled casein was measured with a liquid scintillation spectrometer. • control (absence of quercetin); △, 30 μM quercetin.

Table 1. The ID₅₀ of Seven Natural Anti-oxidative Substances, GA and oGA on the CK-II-Mediated Phosphorylation of P Proteins in Vitro

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>ID₅₀ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin</td>
<td>2.0</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.05</td>
</tr>
<tr>
<td>EGCG</td>
<td>0.3</td>
</tr>
<tr>
<td>Genistein</td>
<td>20.0</td>
</tr>
<tr>
<td>4',7,8,THI</td>
<td>1.5</td>
</tr>
<tr>
<td>3',4',7,THI</td>
<td>0.4</td>
</tr>
<tr>
<td>8C-3',4',5,7-THI</td>
<td>0.1</td>
</tr>
<tr>
<td>oGA</td>
<td>1.0</td>
</tr>
<tr>
<td>GA</td>
<td>80.0</td>
</tr>
</tbody>
</table>

The inhibitory effects of the indicated compounds on CK-II-mediated phosphorylation of P proteins were determined after incubation (20 min at 30°C) with [γ-32P]ATP and poly-Arg. Mean values (ID₅₀) from three different experiments (CK-II-mediated phosphorylation of p33 (P0) inhibited by the indicated compounds).

CK-II activity was observed. In our experimental system of the CK-II-mediated phosphorylation of 60S acidic ribosomal P proteins, quercetin inhibited 50% inhibition of CK-II activity at approx. 50 nM, which is about one-fifth of the concentration required to inhibit the tyrosine-kinase activity of the EGF receptor.30 Taken together, all these observations suggest that CK-II may play an important role in the physiological regulation of the 60S acidic ribosomal P proteins in protein synthesis; (i) natural polyphenol-containing anti-oxidative compounds (quercetin, EGCG and isoflavones) act as suppressors of CK-II activity; and (ii) GL and GA at low levels (0.1—1 μM) significantly stimulate the CK-II-mediated phosphorylation of cellular functional proteins in growing plant cells. Since the 60S acidic ribosomal P proteins are not absolutely necessary for accurate protein synthesis but are also required for certain aspects of growth and developmental differentiation in plant cells,30 it will be important to elucidate the regulatory mechanism of CK-II-mediated phosphorylation of the ribosomal P proteins by natural potent CK-II inhibitors with polyphenols in vivo.

Acknowledgments This work was supported in part by...
grants from Kitasato University (SAHS-A102, 1998) and the Ministry of Education, Science, Sports and Culture of Japan (Grant-in-Aid No. 10670424, 1998). We are grateful to Dr. Ian Gleadall for critical comments on the manuscript.

REFERENCES