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The Effect of pH and Various Cations on the GTP Hydrolysis of Rice Heterotrimeric G-protein α Subunit Expressed in *Escherichia Coli*

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Previously, we reported the biochemical properties of RGA1 that is expressed in *Escherichia coli* (Seo *et al.*, 1997). The activities of RGA1 that hydrolyzes and binds guanine nucleotide were dependent on the $MgCl_2$ concentration. The steady state rate constant (k_{cat}) for GTP hydrolysis of RGA1 at 2 mM $MgCl_2$ was 0.0075 ± 0.0001 min^{-1}. Here, we examined the effects of pH and cations on the GTPase activity. The optimum pH at 2 mM $MgCl_2$ was approximately 6.0; whereas, the pH at 2 mM NH_4Cl was approximately 4.0. The result from the cation dependence on the GTPase (guanosine 5'-triphosphatase) activity of RGA1 under the same condition showed that the GTP hydrolysis rate (k_{cat} = 0.0353 min^{-1}) under the condition of 2 mM NH_4Cl at pH 4.0 was the highest. It corresponded to about 3.24-fold of the k_{cat} value of 0.0109 min^{-1} in the presence of 2 mM $MgCl_2$ at pH 6.0.

Keywords: Cations, GTPase activity, GTP hydrolysis rate (k_{cat}), pH, RGA1

Introduction

The heterotrimeric guanine nucleotide-binding regulatory proteins (G-proteins) comprise a superfamily of proteins (Freissmuth *et al*., 1989; Birnbaumer *et al*., 1990; Bourne *et al*., 1990) that serve to transduce and amplify the signals, which are initially perceived by integral plasma membrane receptor proteins (Im, 2001).

Since early in the 1990s, there has been a steady accumulation of biochemical data, which indicated that heterotrimeric G-proteins might exist in plants, like tobacco, maize, peas, soybeans, and arabidopsis, etc. (Warpeha *et al*., 1991; Legendre *et al*., 1992; Wise and Millner, 1992; Weiss *et al*., 1993; Wise *et al*., 1993). However, most of the evidence was indirect ones, by mainly using a nonhydrolyzable analogue of GTP, GTPγS, or ADP-ribosylation. In addition, not only the genes that encode heterotrimeric Gα subunit homologues from rice, arabidopsis, tomato, *Lotus japonicus* and soybeans, etc. (Ma *et al*., 1990; Ma *et al*., 1991; Poulsen *et al*., 1994; Kim *et al*., 1995; Seo *et al*., 1995), but also those that encode Gβ subunit homologues from maize, arabidopsis, tobacco, and rice (Isida *et al*., 1993; Weiss *et al*., 1994; Ishkawa *et al*., 1996) were isolated. Animal counterparts (such as K^+ channels, stomatal opening, Ca^{2+}/CaM-dependent, and -independent phytochrome signaling, etc.) are known to be regulated by G-proteins. These have been intensively studied by many researchers (Lee *et al*., 1993; Neuhaus *et al*., 1993; Millner, 1996; Park *et al*., 2000). From their comparative studies, it was suggested that heterotrimeric G-proteins in plants may be analogous to those in animal cells.

Furthermore, even though little progress has been made in identifying the receptors and effectors that activate or respond to heterotrimeric G-proteins, possible candidates for cytosolic effectors that are involved in signal transduction in plants have been proposed. However, they have not been fully characterized. For example, in the case of cultured-soybean cells using the antigen-binding fragment (Fab) of an antibody against G-protein, the mastoparan and A subunit of cholera toxin, Legendre *et al.* (1993) demonstrated that G-proteins were involved in the elicitation of the defense responses. In addition, Li and Assmann (1993) reported that *Viva faba* G-proteins took part in the regulation of the outward K^+ channel activity.

Previously, we reported through photoaffinity labeling and GTPγS binding assays that RGA1 expressed in *E. coli* had specific binding properties for guanine nucleotide. Also, varying applications of the $MgCl_2$ concentrations (over a range of 0.5-200 mM) revealed that the rate of GTP

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hydrolysis was the highest at 2 mM $MgCl_2$ (Seo *et al*., 1997).

In the present work, on the basis of the released amounts of Pi and k_{cat} values, we investigated the effects of pH and various cations on the GTPase activity of the rice heterotrimeric G-protein α subunit (RGA1) that is expressed in *E. coli*. Our data indicated that GTPase activity of RGA1 in the presence of a monovalent cation, NH_4Cl, was the highest; pH was also an important parameter in regulating the RGA1 activity.

Materials and Methods

Chemicals Ni^{2+}-NTA (nickel-nitrilotriacetic acid) agarose resin was purchased from Qiagen (Hilden, Germany). The PEI(polyethyleneimine)-cellulose-TLC(thin layer chromatography) reagents were from Aldrich (Milwaukee, USA). The [α-^{32}P]GTP and [γ-^{32}P]GTP (3,000 Ci/mmol) were obtained from Amersham Pharmacia Biotech (Buckinghamshire, England). Most of other reagents came from Sigma (St. Louis, USA) and GIBCO BRL Life Technologies (Groningen, Netherlands).

Purification of the RGA1 overexpressed in *Escherichia coli* RGA1 cDNA fragments were cloned into the pRSET B vector (Seo *et al*., 1997). The *E. coli* strain BL21/DE3(pLysS) was transformed with recombinant plasmids (pRGA1A), and the protein (RGA1) was induced in *E. coli* with 0.5 mM IPTG (isopropyl-β-D-thiogalactopyranoside) for 3 h at 28°C. The cells were pelleted, resuspended, and lysed by the freeze-thaw method. The lysate was centrifuged, and the supernatant was recovered and applied directly to a Ni^{2+}-NTA agarose column. The following steps after purification were carried out as described in a previous work by Seo *et al*. (1997). The purified proteins were dialyzed against the HEDL [50 mM Hepes/NaOH, pH 8.0, 1 mM EDTA, 3 mM DTT, 0.05% $C_{12}E_{10}$ (polyoxyethylene 10-lauryl ether)] buffer that contained 0.5 μM GDP and 30 μM $MgCl_2$.

GTPase assay The GTPase assay, using TLC, was carried out by a slightly modified method of Wagner *et al*. (1987). Briefly, each reaction was proceeded in 100 μl of the HEDL buffer that contained 2 mM each of various cations, 1 mM NaN$_3$, 33 nM [α-^{32}P]GTP (3000 Ci/mmol), 1 mM ATP, and 250 ng of the RGA1 protein at 30°C. Ten microliter aliquots were sampled at appropriate time intervals and added to 10 μl of 0.5 M EDTA (pH 8.0) to stop the reaction. Two microliters were spotted onto a PEI-cellulose TLC plate, which was then developed in a 0.5 M KH_2PO_4 (pH 3.4) solution. After drying, the plate was exposed to X-ray film. GTPase activity was also measured by using charcoal, as previously described in detail (Brandt and Ross, 1985). Briefly, RGA1 (250 ng) was incubated in the HEDL buffer that contained 0.2 μM [γ-^{32}P]GTP at 30°C. For the measurement of time-dependent released amounts of '^{32}P', the reaction was initiated by additions of the protein and 2 mM each of various cations to the mixture that was pre-warmed to the reaction temperature. Twenty-five microliter aliquots were removed at the indicated time intervals, added to 775 μl of 5% (w/v) charcoal in 50 mM NaH_2PO_4 on ice, and vortexed. The charcoal was removed by centrifugation at 2,000× *g* for 15 min. The total amounts of radioactivity in a 400 μl aliquot of the supernatant were measured in a LSC (liquid scintillation counter). In order to study the pH effect in the presence of various cations, the reaction was carried out in the range of pH 1.0 - 12.0. Various pHs of the reaction mixture were adjusted by careful titrations with HCl or NaOH, as required. Data shown in Fig. 1 and 3 are the means of three independent experiments. Those shown in Table 1 are the mean values of triplicate determinations per a single experiment. All of the values and drawings were calculated and plotted by using the computer program "Softwindow Grafit".

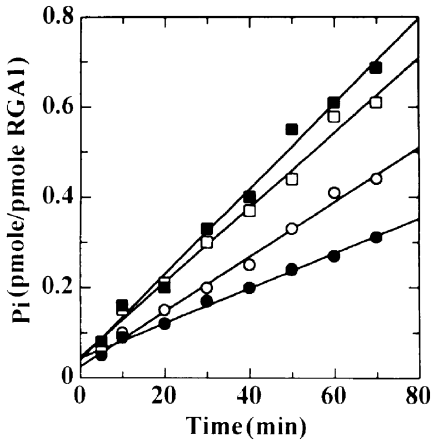

Fig. 1. GTPase activity of RGA1 in a steady state. RGA1 proteins (250 ng) were incubated with 0.2 μM [γ-^{32}P]GTP at 30°C, and one of 2 mM NaCl (□), 2 mM $MnCl_2$ (●), 2 mM $MgCl_2$ (○), or 2 mM NH_4Cl (■). Aliquots (25 μl) were withdrawn at the indicated times, and the released '^{32}Pi' were determined as described in Materials and Methods. Data represent mean values obtained from three independent measurements.

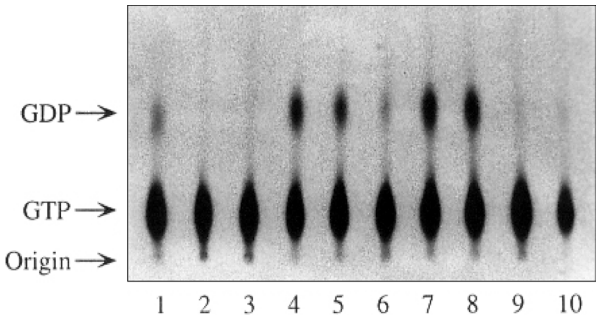

Fig. 2. The effects of various cations on the GTPase activity of RGA1 at pH 8.0. RGA1 (250 ng) were incubated with 33 nM [α-^{32}P]GTP for 3 h at 30°C in the presence of 2 mM concentrations of various cations. Aliquots from the reaction mixture were withdrawn, separated on a PEI-cellulose TLC plates, and visualized by autoradiography, as described in Materials and Methods. Lane 1, $CaCl_2$; lane 2, $CuCl_2$; lane 3, $FeCl_2$; lane 4, KCl; lane 5, $MgSO_4$; lane 6, $MnCl_2$; lane 7, NaCl; lane 8, NH_4Cl; lane 9, $NiCl_2$; lane 10, $ZnCl_2$.

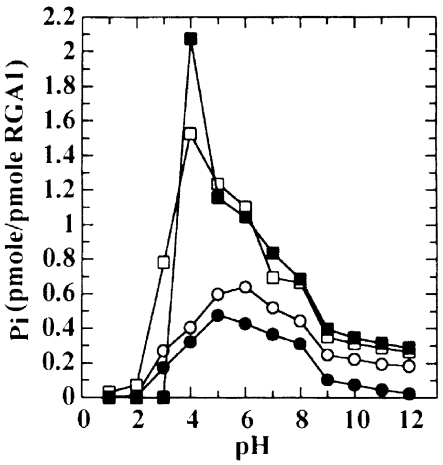

Fig. 3. The pH dependence of the GTPase activity of RGA1 RGA1 (250 ng) was incubated with 0.2 μM [γ-^{32}P]GTP for 70 min at 30°C in the presence of cations at various pHs. After incubation, the released amounts of '^{32}Pi' were determined as described in Materials and Methods. The symbols used are the same as in Figure 1. Data represent mean values that were obtained from three independent measurements.

Table 1. Optimum pH and the rate constants for GTP hydrolysis of RGA1 in the presence of various cations. Protein (250 ng) was incubated for 70 min at 30°C in a final volume of 100 μl with 0.2 μM [γ-^{32}P]GTP and 2 mM concentration of various cations at fixed pH. The rates (k_{cat}) were determined by using the computer program "Softwindow Grafit".

	Optimum pH	Intrinsic GTPase activity k_{cat} (min^{-1})
$CaCl_2$	6	0.0101
$CuCl_2$	5	0.0002
$FeCl_2$	5	0.0031
KCl	4	0.0290
$MgCl_2$	6	0.0190
$MnCl_2$	5	0.0081
NaCl	4	0.0259
NH_4Cl	4	0.0353
$NiCl_2$	5	0.0024
$ZnCl_2$	ND*	0.0085×10^{-2}

*; ND means that optimum pH was not observed.

Results and Discussion

We previously reported that a rice cDNA of the G-protein α subunit was expected to encode a polypeptide of an approximate molecular mass of 44.5 kDa, and the His-tag fused RGA1 protein had an estimated size of 49 kDa. By using a Ni^{2+}-NTA column the His-tag-bearing RGA1 expressed in *E. coli* BL21/DE3 (pLysS) could be highly purified according to the previously reported methods (Seo *et al*., 1997).

Most of the heterotrimeric G-protein α subunits are known to have intrinsic GTPase activities. We also examined the GTPase activity of RGA1 in changing GTP to GDP, and confirmed that the maximal GTP binding and hydrolysis of RGA1 occurred at a 2 mM $MgCl_2$ concentration. Varying applications of the $MgCl_2$ concentration over a range of 0.5 to 200 mM revealed that the rate of GTPase activity was the highest at 2 mM $MgCl_2$. The k_{cat} value was 0.0075 ± 0.0001 min^{-1} in the presence of 2 mM $MgCl_2$ (Seo *et al*., 1997).

Generally, the GTP- and ATP-hydrolyzing proteins have absolute requirements for divalent cations, usually Mg^{2+}, as a cofactor in their reactions. Schweins *et al*.(1997) also reported that in case of the catalytic rate of $p21^{ras}$, a GTP hydrolyzing protein needed the divalent cation of Mg^{2+} in its activity. In addition, they presented that Mn^{2+} was able to efficiently substitute Mg^{2+} in the active site. In 1993, Bertchtold *et al*. and Kjeldgaad *et al*. presented similar results on EF-Tu through their crystal structure studies.

In plants, in order to know whether metal ions affect the GTPase activity of RGA1, we first separately examined it in the presence of a 2 mM concentration of $MgCl_2$, $MnCl_2$, NaCl, and NH_4Cl. As shown in Fig. 1, the hydrolyzed "Pi" amounts gradually increased with the incubation time under all the conditions. We also checked the effects of monovalent or divalent cations on the GTP hydrolysis of RGA1 at pH 8.0. Of all the cations that were tested, we confirmed that the hydrolysis activity of GTP to GDP of RGA1 was relatively high both in NH_4Cl, KCl, and NaCl among the monovalent cations, and in $MgCl_2$, $CaCl_2$, and $MnCl_2$ among the divalent cations (Fig. 2). However, contrary to our results, Northup *et al*. (1982) reported by using the regulatory component of adenylate cyclase that the divalent cations (such as Mg^{2+}, Mn^{2+} and Ca^{2+} except Fe^{2+}, Zn^{2+} and Cu^{2+}) were all effective for guanine nucleotide-binding. In addition, Wise *et al*. (1997) also proposed that the GTPγS binding of Arabidopsis GPα1(G-protein α subunit 1) that is expressed in *E. coli* was stimulated about 2-fold in the presence of Zn^{2+} when compared to that in the presence of Mg^{2+}, Mn^{2+}, or Ca^{2+}. In case of $p21^{ras}$, the GTPase reaction accelerated to 4.4-fold, when Mg^{2+} was replaced by Mn^{2+}. However, neither Zn^{2+} nor Cd^{2+} was able to replace the strongly bound Mg^{2+} (Schweins *et al*., 1997). Also, similar results were shown for EF-Tu, since Mn^{2+} was able to efficiently substitute Mg^{2+} in the active site, and Mn^{2+} increased the GTPase rate of EF-Tu several-fold, depending on the conditions (Kalbitzer *et al*., 1990; Kreb and Parmeggiani, 2002; Wieden *et al*., 2002). Therefore, a more detailed structural analysis is needed in order to understand their exact role in the catalytic mechanism.

It is reasonable to assume from some reports that the distinct catalytic efficiencies are caused by the stability of the transition state of each reaction by various metal ions. In 1995, Schweins *et al*. demonstrated that the apparent pKa of the γ-phosphate of protein-bound GTP could be determined by fitting the increase in a reaction rate below pH 7.0. Therefore, this immediately led us to examine the pH effect of the GTPase activity of RGA1. It

was investigated with the same cations that were tested in Fig. 1 in the range of pH 1.0 - 12.0. The GTPase activity of RGA1 was the highest at pH 6.0 in the presence of $MgCl_2$, pH 5.0 in the presence of $MnCl_2$, and pH 4.0 in the presence of NaCl or NH_4Cl. Furthermore, we conducted additional experiments to confirm the optimum pH for the GTPase activity of RGA1 by six other cations (such as $CaCl_2$, $CuCl_2$, $FeCl_2$, KCl, $NiCl_2$, and $ZnCl_2$) under the same conditions as shown in Fig. 3. From these experiments, we calculated the GTP hydrolysis rates (k_{cat}) of RGA1 in the presence of 2 mM concentration of each cation and optimum pH condition. As shown in Table 1, the k_{cat} value was the highest among the reaction conditions as 0.0353 min^{-1} in the presence of NH_4Cl at pH 4.0. This value was approximately 3.24-fold of the k_{cat} value (0.0109 min^{-1}) in the presence of $MgCl_2$ at pH 6.0. In addition, the GTPase activity of RGA1 in the presence of 2 mM concentrations of the cations used at pH 8.0 varied (the highest with K^+, Na^+, and NH_4^+; slightly higher with Ca^{2+} and Mg^{2+}; medium with Mn^{2+}; low with Fe^{3+} and Ni^{2+}; very low with Cu^{2+} and Zn^{2+}).

Recently, a few reports on the determination of crystal structures significantly contributed to our understanding at a molecular level of the physiological roles of heterotrimeric G-proteins in the signaling process. The role of metal ion for these proteins was particularly well documented through structural considerations. (Sondek *et al*., 1994; Mixon *et al*., 1995; Wall *et. al*., 1995; Lambright *et al*., 1996). Furthermore, a novel reaction mechanism for guanine nucleotide-binding proteins, which is based on theoretical, structural, and functional considerations, was proposed (Muegge *et al*., 1998; Coleman and Sprang, 1999; Hanzal-Bayer *et al*., 2002).

Elucidation of the RGA1 crystal structure may be a very important step towards a more detailed understanding of the hydrolysis mechanism of this protein in the presence of various cations and under different pHs.

In conclusion, we demonstrated here that a rice heterotrimeric GTP-binding protein (RGA1) that is expressed in *E. coli* had cation- and pH-dependent properties for GTPase activity. NH_4Cl (among the tested monovalent cations) and $MgCl_2$ (among the tested divalent cations) were particularly effective for GTP hydrolysis at pH 4.0 and pH 6.0, respectively.

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References

Birnbaumer, L., Abramowitz, J. and Brown, A. M. (1990) Receptor-effector coupling by G proteins. *Biochim. Biophys. Acta* **1031**, 163-224.

Bourne, H. R., Sanders, D. A. and McCormick, F. (1990) The GTPase superfamily: a conserved switch for diverse cell functions. *Nature* **348**, 125-132.

Brandt, D. R. and Ross, E. M. (1985) GTPase activity of the stimulatory GTP-binding regulatory protein of adenylate cyclase, Gs. Accumulation and turnover of enzyme-nucleotide intermediates. *J. Biol. Chem.* **260**, 266-272.

Coleman, D. E. and Sprang, S. R. (1999) Structure of Gialpha1.GppNHp, autoinhibition in a galpha protein-substrate complex. *J. Biol. Chem.* **274**, 16669-16672.

Freissmuth, M., Casey, P. J. and Gilman, A. G. (1989) G proteins control diverse pathways of transmembrane signaling. *FASEB J.* **3**, 2125-2131.

Hanzal-Bayer, M., Renault, L., Roversi, P., Wittinghofer, A. and Hillig, R. C. (2002) The complex of Arl2-GTP and PDE delta: from structure to function. *EMBO J.* **21**, 2095-2106.

Im, M.-J. (2001) Regulation of a novel guanine nucleotide binding protein tissue transglutaminase (Gah). *J. Biochem. Mol. Biol.* **34**, 95-101.

Isida, S., Takahashi, Y. and Nagata, T. (1993) Isolation of cDNA of an auxin-regulated gene encoding a G protein beta subunit-like protein from tobacco BY-2 cells. *Proc. Natl. Acad. Sci. USA* **90**, 11152-11156.

Ishikawa, A., Iwasaki, Y. and Asahi, T. (1996) Molecular cloning and characterization of a cDNA for the beta subunit of a G protein from rice. *Plant Cell Physiol.* **37**, 223-228.

Kalbitzer, H. J., Feuerstein, J., Goody, R. S. and Wittinghofer, A. (1990) Stereochemistry and lifetime of the GTP hydrolysis intermediate at the active site of elongation factor Tu from Bacillus stearothermophilus as inferred from the 17O-55Mn superhyperfine interaction. *Eur. J. Biochem.* **188**, 355-359.

Krab, I. M. and Parmeggiani, A. (2002) Mechanisms of EF-Tu, a pioneer GTPase. *Prog. Nucleic Acid Res. Mol. Biol.* **71**, 513-551.

Kim, W. Y., Cheong, N. E., Lee, D. C., Je, D. Y., Bahk, J. D., Cho, M. J. and Lee, S. Y. (1995) Cloning and sequencing analysis of a full-length cDNA encoding a G protein alpha subunit, SGA1, from soybean. *Plant Physiol.* **108**, 1315-1316.

Lambright, D. G., Sondek, J., Bohm, A., Skiba, N. P., Hamm, H. E. and Sigler, P. B. (1996) The 2.0 A crystal structure of a heterotrimeric G protein. *Nature* **379**, 311-319.

Lee, H. J., Tucker, E. B., Crain, R. C. and Lee, Y. S. (1993) Stomatal opening is induced in epidermal peels of *Commelina communis* L. by GTP analogs or pertussis toxin. *Plant Physiol.* **102**, 95-100.

Legendre, L., Heistein, P. F. and Low, P. S. (1992) Evidence for participation of GTP-binding proteins in elicitation of the rapid oxidative burst in cultured soybean cells. *J. Biol. Chem.* **267**, 20140-20147.

Legendre, L., Yueh, Y. G., Crain, R., Haddock, N., Heinstein, P. F. and Low, P. S. (1993) Phospholipase C activation during elicitation of the oxidative burst in cultured plant cells. *J. Biol. Chem.* **268**, 24599-24563.

Li, W. and Assmann, S. M. (1993) Characterization of a G-protein-regulated outward potassium current in mesophyll cells of *Vicia faba*. *Proc. Natl. Acad. Sci. USA* **91**, 262-266.

Ma, H., Yanofsky, M. F. and Meyerowitz, E. M. (1990) Molecular cloning and characterization of GPA1, a G protein alpha subunit gene from *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* **87**, 3821-3825.

Ma, H., Yanofsky, M. F. and Huang, H. (1991) Isolation and sequence analysis of TGA1 cDNAs encoding a tomato G protein alpha subunit. *Gene* **107**, 189-195.

Millner, P. A. and Causier, B. A. (1996) G-protein-coupled receptors in plant cells. *J. Exp. Bot.* **47**, 983-992.

Mixon, M. B., Lee, E., Colemal, D. E., Berghuis, A. M., Gilman, A. G. and Sprang, S. R. (1995) Tertiary and quaternary structural changes in Gi alpha 1 induced by GTP hydrolysis. *Science* **270**, 954-960.

Muegge, I., Schweins, T. and Warshel, A. (1998) Electrostatic contributions to protein-protein binding affinities: application to Rap/Raf interaction. *Proteins* **30**, 407-423.

Neuhaus, G., Bowler, C., Kerm, R. and Chua, N. H. (1993) Calcium/calmodulin-dependent and -independent phytochrome signal transduction pathways. *Cell* **73**, 937-952.

Northup, J. K., Smigel, M. D. and Gilman, A. G. (1982) The guanine nucleotide activating site of the regulatory component of adenylate cyclase. Identification by ligand binding. *J. Biol. Chem.* **257**, 11416-11423.

Park, S., Nelson, T. J., Alkon, D. L. and Kim, J. (2000) Functional characterization of the squid calexcitin-2, a calcium and GTP-binding protein. *J. Biochem. Mol. Biol.* **33**, 391-395.

Poulsen, C., Mai, X. M. and Borg, S. (1994) A *Lotus japonicus* cDNA encoding an alpha subunit of a heterotrimeric G-protein. *Plant Physiol*. **105**, 1453-1454.

Schweins, T., Scheffzek, K., Aâheuer, R. and Wttinghofer, A. (1997) The role of the metal ion in the p21ras catalysed GTP-hydrolysis: Mn^{2+} versus Mg^{2+}. *J. Mol. Biol.* **266**, 847-856.

Seo, H. S., Choi, C. H., Lee, S. Y., Cho, M. J. and Bahk, J. D. (1997) Biochemical characteristics of a rice (*Oryza sativa* L., IR36) G-protein alpha-subunit expressed in *Escherichia coli*. *Biochem. J.* **324**, 273-281.

Seo, H. S., Kim, H. Y., Jeong, J. Y., Lee, S. Y., Cho, M. J. and Bahk, J. D. (1995) Molecular cloning and characterization of RGA1 encoding a G protein alpha subunit from rice (*Oryza sativa* L. IR-36). *Plant Mol. Biol.* **27**, 1119-1131.

Sondek, J., Lambright, D. G., Noel, J. P., Hamm, H. E. and Sigler, P. B. (1994) GTPase mechanism of Gproteins from the 1.7-A crystal structure of transducin alpha-GDP-AIF-4. *Nature* **372**, 276-279.

Wagner, P., Molenaar, C. M. T., Rauh, A. G. J., Brokel, R., Smith, H. D. and Gallwitz, D. (1987) Biochemical properties of the ras-related YPT protein in yeast: a mutational analysis. *EMBO J.* **6**, 2373-2379.

Wall, M. A., Coleman, D. E., Lee, E., Iniguez-Lluhi, J. A., Posner, B. A., Gilman, A. G. and Sprang, S. R. (1995) The structure of the G protein heterotrimer Gi alpha 1 beta 1 gamma 2. *Cell* **83**, 1047-1058.

Warpeha, K. M., Hamm, H. E., Rasenick, M. M. and Kaufman, L. S. (1991) A blue-light-activated GTP-binding protein in the plasma membranes of etiolated peas. *Proc. Natl. Acad. Sci. USA* **88**, 8925-8929.

Weiss, C. A., Garnaat, C. W., Mukai, K., Hu, Y. and Ma, H. (1994) Isolation of cDNAs encoding guanine nucleotide-binding protein beta-subunit homologues from maize (ZGB1) and Arabidopsis (AGB1). *Proc. Natl. Acad. Sci. USA* **91**, 9554-9558.

Weiss, C. A., Huang, H. and Ma, H. (1993) Immunolocalization of the G protein alpha subunit encoded by the GPA1 gene in Arabidopsis. *The Plant Cell* **5**, 1513-1528.

Wieden, H. J., Gromadski, K., Rodnin, D. and Rodnina, M. V. (2002) Mechanism of elongation factor (EF)-Ts-catalyzed nucleotide exchange in EF-Tu. Contribution of contacts at the guanine base. *J. Biol. Chem.* **277**, 6032-6036.

Wise, A. and Millner, P. A. (1992) Evidence for the presence of GTP-binding proteins in tobacco leaf and maize hypocotyl plasmalemma. *Biochem. Soc. Trans.* **20**, 7.

Wise, A., Thomas, P. G., Murphy, G. A. and Millner, P. A. (1997) Expression of the Arabidopsis G-protein GP alpha1: purification and characterization of the recombinant protein. *Plant Mol. Biol.* **33**, 723-728.

Wise, A., White, I. R. and Millner, P. A. (1993) Stimulation of guanosine 5'-O-(3-thio)triphosphate binding to higher plant plasma membranes by the mastoparan-analogue, Mas 7. *Biochem. Soc. Trans.* **21**, 228.