J. Biochem. Mol. Biol. Vol. 28, No. 3 pp. 197~203 May 31, 1995

Purification and Characterization of Farnesyl Protein Transferase from Bovine Testis

Kwon-Yul Ryu, Young-Jin Baik and Chul-Hak Yang*

Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151-742, Korea

(Received August 20, 1994)

Abstract: Farnesyl protein transferase involved in the first step of post-translational modification of $p21^{ras}$ proteins transfers the farnesyl moiety from farnesyl pyrophosphate to a cysteine residue in $p21^{ras}$ proteins. The enzyme was first purified 30,000-fold from bovine testis by use of 30~50% ammonium sulfate fractionation, DEAE-Sephacel ion exchange chromatography, Sephacryl S-300 gel filtration chromatography, Sephacryl S-200 gel filtration chromatography, and hexapeptide (Lys-Lys-Cys-Val-Ile-Met) affinity chromatography. The molecular weight of the purified enzyme was estimated to be ~100 kDa by gel filtration and SDS-polyacrylamide gels showed two closely spaced bands of ~50 kDa protein. These indicate that the enzyme consists of two nonidentical subunits, α and β, which have slightly different molecular weights. The enzyme was inhibited by hexapeptide (Lys-Lys-Cys-Val-Ile-Met), which acted as an alternative substrate that competed for farnesylation. Kinetic analysis by measuring initial velocities showed that farnesyl protein transferase is a very slow enzyme. EDTA-treated farnesyl protein transferase showed little activity with Mg^{2+} or Zn^{2+} alone, but required both Mg^{2+} and Zn^{2+} for the catalytic activity.

Key words: Farnesyl protein transferase, bovine testis, hexapeptide (Lys-Lys-Cys-Val-Ile-Met) affinity chromatography, p^{21ras} proteins.

Farnesyl protein transferase (FPT) is a key enzyme that is responsible for the first step of post-translational modification of various cellular proteins. These proteins include $p21^{ras}$ proteins, nuclear lamins A and B, yeast a-factor, and the γ-subunit of transducin (Clarke, 1992). A common feature of these proteins is the presence of the C-terminal "CAAX-motif", where C is a cysteine residue to which the farnesyl group is attached, A is an aliphatic amino acid, and X can be either methionine, serine, phenylalanine, or alanine (Reiss *et al.*, 1991a). Farnesylation is followed by proteolytic cleavage of the three C-terminal amino acids ("AAX") and methylation of the carboxyl group of the cysteine residue (Gibbs, 1991). These three steps make the C-terminus of protein highly hydrophobic, and promote membrane interactions (Casey, 1992). Another enzyme, geranylgeranyl protein transferase, recognizes the proteins which have the C-terminal sequences such as CAAL, where L is a leucine, CXC, or CC, and directs geranylgeranyl addition (Seabra *et al.*, 1991; Moores *et al.*, 1991; Kinsella *et al.*, 1991).

By assaying its ability to farnesylate $p21^{ras}$ proteins *in vitro*, FPT has been purified to homogeneity from either rat or bovine brain (Reiss *et al.*, 1990; Moores *et al.*, 1991). The enzyme is a heterodimer composed of α-subunit (49 kDa) and β-subunit (46 kDa), both of which are required for catalytic activity (Reiss *et al.*, 1990, 1991b). At present, it is known that the α- and β-subunits are the mammalian homologues of the RAM 2 and the DPR1/RAM1 gene products, respectively, of yeast (Chen *et al.*, 1991; He *et al.*, 1991). FPT recognizes even a short sequence of only four peptides corresponding to the C-terminal sequences of known farnesylated proteins (Reiss *et al.*, 1990). This property aided its purification by peptide affinity chromatography. Chemical cross-linking studies have shown that the β-subunit is responsible for the peptide-binding (Reiss *et al.*, 1991b). It was postulated that the α-subunit performs its function by binding to the farnesyl pyrophosphate (FPP) (Reiss *et al.*, 1991b).

FPT shows a requirement for divalent cations, Mg^{2+} and Zn^{2+} (Reiss *et al.*, 1992). It is thought that Mg^{2+} acts as an activator for the pyrophosphate group to leave FPP and catalyzes the transfer of the farnesyl group to the bound peptide substrate (Moomaw *et al.*, 1992). The tightly bound Zn^{2+} exerts its effects at the level of peptide substrate binding, which was proved

*To whom correspondence should be addressed.
Tel: 82-2-878-8545, Fax: 82-2-878-8545.

by the glutaraldehyde cross-linking of peptide substrate to FPT (Reiss *et al.*, 1992). A steady-state kinetic analysis has shown that substrate binding to the enzyme occurs in random order (Pompliano *et al.*, 1992). This analysis is consistent with the ability of the enzyme to bind to a peptide affinity column in the absence of FPP, and to form a stable complex with FPP in the absence of a protein substrate (Reiss *et al.*, 1990, 1991b).

In this paper, we first report the purification and characterization of FPT from bovine testis and document it as a good source for identifying the active site, determining the structure, and producing the inhibitor that can block farnesylation of ras proteins without affecting the other pathways.

Materials and Methods

Materials

[1-^{3}H]Farnesyl pyrophosphate ([^{3}H]FPP, 22.5 Ci/mmol) was purchased from Du Pont-New England Nuclear. Hexapeptide (KKCVIM) was custom synthesized by Korea Basic Science Center. Glutathione S-transferase-fused undecapeptide (Gly-Cys-Val-Lys-Ile-Lys-Lys-Cys-Val-Ile-Met), which has a C-terminal sequence of $p21^{Ki\text{-}ras}$, was produced in *E. coli* and purified. DEAE-Sephacel, Sephacryl S-300, and Sephacryl S-200 were purchased from Pharmacia-LKB Biotechnology, Inc. Activated CH-Sepharose 4B, glutathione-agarose, dithiothreitol (DTT), n-octyl β-D-glucopyranoside, Zwittergent 3-14, HEPES free acid, Trizma base were from Sigma. NaCl, $(NH_4)_2SO_4$, SDS were from Merck. EDTA was from Aldrich. All other chemicals and reagents used were of commercial reagent grade.

Purification of glutathione S-transferase fused undecapeptide (GST-PEP)

pGEX-2T vector was used to make the recombinant DNA, pGPEP, which expressed undecapeptide (GCVKIKKCVIM) as fusions with glutathione S-transferase in *E. coli* (Baik, 1994). The *E. coli* containing pGPEP was cultured overnight in an LB/ampicillin medium at 37℃ and induced by 0.1 mM IPTG for 5 h. Cells were harvested and resuspended in a PBS buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 \cdot 7H_2O$, 1.4 mM KH_2PO_4). Cells were lysed by sonication and Triton X-100 was added to a final concentration of 1%. The supernatants were mixed gently with a 50% slurry of glutathione-agarose beads. Beads were washed repeatedly with a PBS buffer, and elution of GST-PEP was carried out by adding 50 mM Tris-Cl (pH 7.5)/5 mM reduced glutathione (Current Protocols in Molecular Biology, unit 16.7). The concentration of GST-PEP was 4.91 μg/ μl and the molecular weight was estimated to be 27 kDa by SDS-PAGE. This fusion protein, GST-PEP, was used as a target protein to which a farnesyl group is transferred.

Assay for farnesyl protein transferase activity

FPT activity assays were carried out by measuring the amount of [^{3}H]farnesyl transferred from [^{3}H]FPP to GST-PEP protein. Unless otherwise noted, each reaction mixture contained the following components in a final volume of 50 μl : 50 mM Na-Hepes (pH 7.5), 100 mM NaCl, 0.2% (w/v) n-octyl β-D-glucopyranoside, 1 mM DTT, 5 μM $ZnCl_2$, 5 mM $MgCl_2$, 7.27 μM GST-PEP, 0.196 μM [^{3}H]FPP (49,950 dpm/pmol), 20~50 ng of affinity-purified enzyme. Assays were performed as described previously (Moores *et al.*, 1991). After incubation at 37℃ for 30 min, the reactions were stopped by addition of 1 M HCl in ethanol (1 ml). To complete the precipitation of [^{3}H]farnesylated GST-PEP and hydrolyze unreacted [^{3}H]FPP, the quenched reaction mixtures were allowed to stand at room temperature for 15 min. After adding 2 ml of 100% ethanol, the mixtures were vacuum-filtered through Whatman 3 MM filters. The filters were washed four times with 2-ml aliquots of 100% ethanol, mixed with Triton X-100 : toluene scintillation fluid (7 ml), and counted in a scintillation counter.

Purification of farnesyl protein transferase

The purification procedures were based on a previous report (Reiss *et al.*, 1990; Moomaw *et al.*, 1992), with some modifications. All steps were performed at 4℃ except step 5, which was done at room temperature.

Step 1-Ammonium sulfate fractionation : Bovine testis (100 g) was homogenized in 60 ml of 50 mM Tris-Cl (pH 7.5) containing 1 mM EDTA, 1 mM EGTA, 0.2 mM PMSF, and 0.42 μM leupeptin, and the extract was centrifuged at 100,000×g for 60 min. The supernatant was saturated to 30% with ammonium sulfate, stirred for 30 min on ice, and centrifuged at 8,000×g for 30 min to remove precipitated proteins. The resulting supernatant was saturated to 50% with ammonium sulfate, and the precipitated proteins were dissolved in 20 ml of 20 mM Tris-Cl (pH 7.5) containing 1 mM DTT and 20 μM $ZnCl_2$, and dialyzed for 4 h against 4 L of the same buffer and then 4 L of a fresh buffer for 12 h.

Step 2-Ion exchange chromatography : The dialyzed 30~50% ammonium sulfate fraction (1.36 g protein) was applied to a DEAE-Sephacel column (3.8×8.2 cm) equilibrated in 50 mM Tris-Cl (pH 7.5) containing 1 mM DTT, 20 μM $ZnCl_2$, and 0.05 M NaCl.

The column was washed and eluted with an 800 ml linear gradient of 0.05~0.50 M NaCl in the same buffer at a flow rate of 60 ml/h. Fractions eluting between 0.24 M and 0.26 M NaCl contained the majority of FPT activity. These fractions (40 ml) were pooled, and concentrated to 4.8 ml by ultrafiltration.

Step 3-Gel filtration chromatography I: 2 ml (45.5 mg protein) of DEAE-purified material was applied to a Sephacryl S-300 column (1.5×54 cm) equilibrated in 50 mM Tris-Cl (pH 7.5) containing 1 mM DTT, 20 μM $ZnCl_2$, and 0.2 M NaCl, and eluted with the same buffer at a flow rate of 6 ml/h. The peak fractions (4 ml) were pooled, and concentrated to 0.45 ml by ultrafiltration.

Step 4-Gel filtration chromatography II: 0.40 ml (3.59 mg protein) of S-300-purified material was applied to a Sephacryl S-200 column (0.9×26 cm) equilibrated in the same buffer as Step 3, and eluted with the same buffer at a flow rate of 2 ml/h. The peak fractions (2 ml) were pooled.

Step 5-Affinity chromatography: An affinity column containing hexapeptide (KKCVIM) corresponding to the C-terminal 6 amino acids of $p21^{Ki\text{-}ras}$ protein was prepared as follows. 0.5 g of activated CH-Sepharose 4B was swollen for 15 min in 100 ml of 1 mM ice-cold HCl on a sintered glass filter and washed with the same solution. 7 mg of the hexapeptide was dissolved in 1 ml of DMSO/dioxane (1 : 1) and 60 μl of 1 M Na-Hepes (pH 8.0) was added, followed by 0.5 ml of 1 M NaCl, 0.42 ml of water, and 20 μl of 1 M DTT to 10 mM. To this cloudy solution, activated CH-Sepharose 4B was added. The resulting mixture was mixed at room temperature for 2 h, followed by overnight incubation with an end-over-end mixing at 4℃. The 1.2 ml slurry was poured into a column, and washed with 1.2 ml of 30% DMSO/dioxane followed by 1.2 ml of water. Excess active groups were blocked by 20 column volume of 0.1 M Tris-Cl (pH 8.0) containing 10 mM DTT at a flow rate of 24 ml/h. Excess uncoupled peptide was removed by ten cycles of alternating washes at the same flow rate, each consisting of a 40-column volume of 0.1 M sodium acetate (pH 4.0) and then 0.1 M Tris-Cl (pH 8.0). Both buffers contained 1 M NaCl and 10 mM DTT. The column was stored at 4℃ in 20 mM Tris-Cl (pH 7.5), 1 mM DTT, and 0.02% NaN_3 until use.

The active enzyme fraction (1.60 mg protein) obtained from Sephacryl S-200 column was applied to a 1.2 ml peptide affinity column equilibrated in 50 mM Tris-Cl (pH 7.5) containing 1 mM DTT and 0.1 M NaCl (buffer A) at a flow rate of 12 ml/h. The enzyme containing solution was cycled back through the column five times. The column was washed with 20 ml of buffer A containing 0.2% (w/v) n-octyl β-D-glucopyranoside (buffer B). The enzyme was eluted with 20 ml of 50 mM Tris-succinate (pH 5.0) containing 1 mM DTT, 0.1 M NaCl, and 0.2% n-octyl β-D-glucopyranoside. This eluate was concentrated to 2 ml and washed twice with 20 ml of buffer B in an ultrafiltration cone and brought to a final volume of 0.73 ml. The affinity-purified enzyme was used in all experiments hereafter.

Treatment of farnesyl protein transferase and GST-PEP with EDTA

To remove metals from affinity-purified FPT and GST-PEP, both proteins were separately treated with EDTA. FPT (0.4 μg in a volume of 40 μl) was mixed with 360 μl of 50 mM Na-Hepes (pH 7.5) containing 1 mM DTT, 0.1 M NaCl, 0.2% (w/v) n-octyl β-D-glucopyranoside, and 0.2 mM EDTA. This mixture was incubated at 4℃ for 1 h and centrifuged at 1,500×g in a Millipore ultrafree-MC filter unit which was coated with 0.5% bovine serum albumin, and brought to 40 μl. To this solution, 360 μl of the same buffer was added. Incubation and centrifugation were repeated twice. GST-PEP (2.46 mg in a volume of 0.5 ml) was dialyzed for 16 h against 500 ml of 20 mM Na-Hepes (pH 7.5) containing 1 mM DTT, 0.1 M NaCl, and 0.2 mM EDTA. EDTA-treated FPT and GST-PEP were used in the study of divalent cation specificities.

Other methods

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by the methods of Laemmli (1970). The protein content of extracts was determined by a Bradford assay or reading absorbance at 280 nm except for that of the affinity-purified material, which was estimated by comparison with a bovine serum albumin (25~200 ng) following SDS-PAGE.

Results and Discussion

FPT was partially purified from various tissues, such as bovine brain, bovine liver, bovine testis, and rat liver. The FPT activities were found in all tissues, but at different levels. Among these, bovine testis had the highest level of activity. Therefore, bovine testis was used as an enzyme source. Fig. 1 shows the elution profile of FPT activity from chromatographic purification steps. The resulting fractions were applied to peptide (KKCVIM) affinity chromatography. Most of the FPT activity was adsorbed to the column, and about 80% of the applied activity was recovered when the column was eluted with a Tris-succinate buffer (pH 5.0). Table 1 summarizes the quantitative results of these purification steps. These results indicate that FPT was purified app-

roximately 30,000-fold with a recovery of 10.2%. Fig. 2a shows protein bands in the silver-stained 10% SDS-polyacrylamide gel at each stage of purification. The peptide affinity column yielded two closely spaced protein bands in approximately equimolar amounts with an apparent subunit molecular weight of ~50 kDa (Fig. 2b). The molecular weight of FPT was estimated to be ~100 kDa by a Sephacryl S-300 column which was calibrated with catalase (232 kDa), collagenase (110 kDa), and bovine serum albumin (66 kDa). Thus, the enzyme is believed to be a heterodimer (α/β), with its subunits having slightly different molecular weights. And both subunits are required for FPT activity. This result is consistent with those of others who purified FPT from rat brain and bovine brain (Reiss *et al.*, 1990; Pompliano *et al.*, 1992).

The dependence of FPT activity on incubation time is shown in Fig. 3. Reaction velocities in assays with a Hepes-buffer were at least 4 times faster than in assays using Tris-buffer (Schaber *et al.*, 1990). The binding of FPT to the peptide affinity column suggested that the enzyme recognized short peptide sequences. In fact, recognition by the enzyme requires only the C-terminal 4 amino acids of the protein (CVIM). As expected, peptide KKCVIM inhibited farnesylation of GST-PEP (Fig. 4). 50% inhibition was observed in the

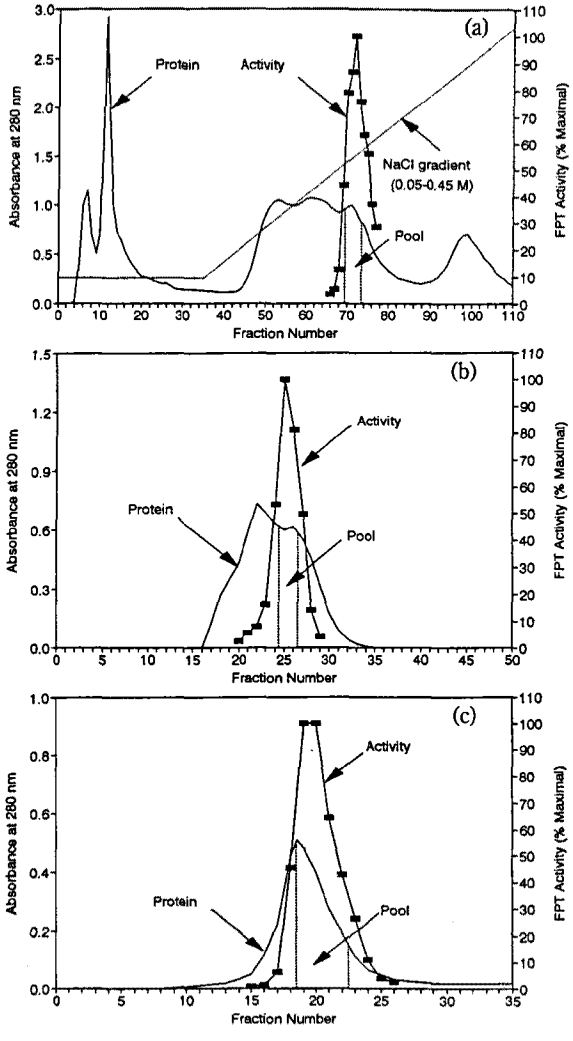

Fig. 1. Chromatographic purification steps of FPT. (a) DEAE-Sephacel ion exchange chromatography, (b) Sephacryl S-300 gel filtration chromatography, (c) Sephacryl S-200 gel filtration chromatography of FPT. 100% FPT activity value was 2.13, 3.58, and 2.71 pmol of [^{3}H]farnesylated GST-PEP formed per 30 min for each step.

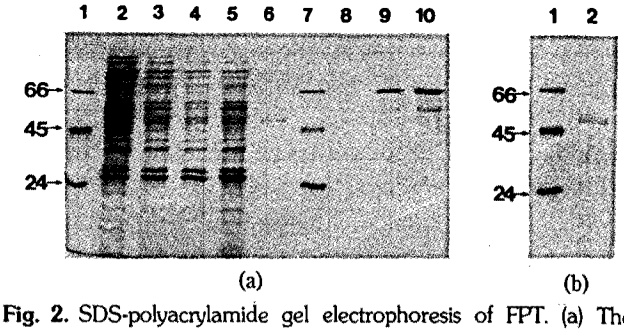

Fig. 2. SDS-polyacrylamide gel electrophoresis of FPT. (a) The various stages of purification. Lane 1, 7 : size markers (66, 45, 24 kDa); Lane 2 : 6 μg of the S-300 fraction; Lane 3 : 4 μg of the S-200 fraction; Lane 4 : unbound fraction of the peptide affinity column; Lane 5 : washing fraction of the peptide affinity column; Lane 6 : peptide affinity column fraction; Lane 8, 9, 10 : 50, 100, 200 ng of bovine serum albumin. (b) Lane 1 : size markers (66, 45, 24 kDa); Lane 2 : approximately 50 ng of purified FPT.

Table 1. Purification of FPT from bovine testis

Fraction	Protein (mg)	Specific activity (U/mg)	Total activity (U)	Purification (fold)	Recovery (%)
Crude extract	2070	0.0410	84.9	1.00	100
30~50% ammonium sulfate	481	0.0825	39.7	2.01	46.8
DEAE-Sephacel	38.5	1.41	54.1	34.4	63.7
Sephacryl S-300	3.42	4.01	13.7	97.8	16.1
Sephacryl S-200	1.60	6.44	10.3	157	12.1
Peptide affinity column	~0.007	1240	8.65	30200	10.2

One unit (U) of enzyme activity is the amount of enzyme that transfers 1 pmol of [^{3}H]farnesyl from [^{3}H]FPP to acid-precipitable GST-PEP per hour.

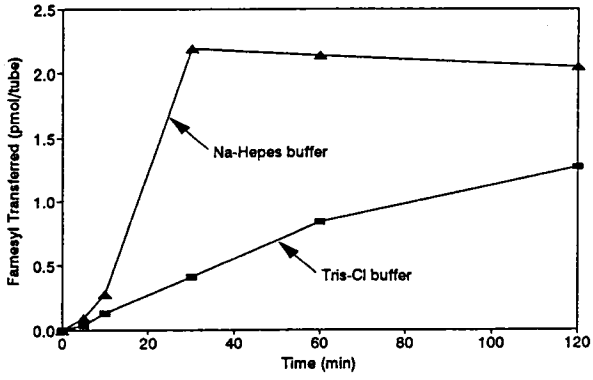

Fig. 3. Dependence of FPT activity on incubation time.

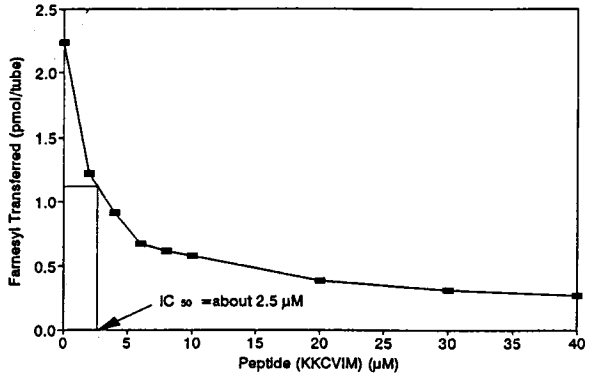

Fig. 4. Inhibition of FPT activity by peptide KKCVIM. Peptide was dissolved at a concentration of 1 mM in DMSO containing 10 mM DTT. All dilutions were made in 10 mM DTT in the absence of DMSO.

presence of 2.5 μM peptide. Fig. 5 shows the ability of the enzyme to tolerate detergents. The inclusion of Zwittergent 3-14 or Triton X-100 in the reactions had no stimulatory effect on the activity of the enzyme. The n-octyl β-D-glucopyranoside stimulated FPT activity even at the concentration of 0.5%, while the bile salt, sodium deoxycholate, was strongly inhibitory to the enzyme even at low concentrations. The enzyme was most active at 50℃ and showed a pH optimum of 7.2.

The acidic ethanol precipitation-filter binding assay helped to improvethe reproducibility of data and lower the background radioactivity (~1/200), probably by better solubilizing [^{3}H]farnesol which is the hydrolyzed product of FPP (Pompliano *et al.*, 1992). With this assay, initial velocities of the reactions were measured accurately. Table 2 shows the kinetic parameters for FPT. An apparent K_M value obtained for GST-PEP protein was 0.623 μM. This value is similar to the previously reported value, 0.63± 0.05 μM, which was obtained for Ras-CVLS, but slightly higher than the value obtained for Ras-CVIM, 0.13± 0.05 μM (Pompliano *et al.*, 1992). Since the GST-PEP and Ras-CVIM have the

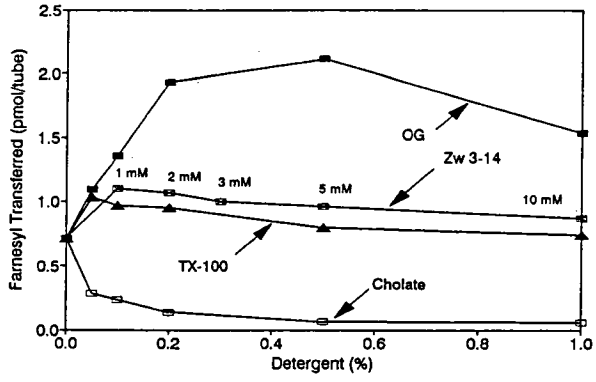

Fig. 5. Effect of detergents on FPT activity. The detergents used were n-octyl β-D-glucopyranoside (OG), Zwittergent 3-14 (Zw 3-14), Triton X-100 (TX-100), and sodium deoxycholate (Cholate).

Table 2. Steady-state kinetic parameters for FPT

Substrate	K_M (μM)	V_{max} (pmol/min)	k_{cat} (min^{-1})	k_{cat}/K_M (M^{-1} min^{-1})
GST-PEP	0.623	0.0274	0.130	2.09×10^5
FPP	0.0366	0.0225	0.107	2.93×10^6

same carboxyl terminal sequences which is recognized by the enzyme, it seems likely that Ras-CVIM is a better substrate than the GST-PEP. The K_M value for FPP was 0.0366 μM. This value is consistent with the previously reported value, 0.04± 0.01 μM (Pompliano *et al.*, 1992). The second-order rate constant, k_{cat}/K_M, is the true measure of the specificity of a substrate for an enzyme. The specificity constant, k_{cat}/K_M, of FPT is much lower than the other enzyme-substrate pairs. So, there is a possibility that allosteric activators control the FPT activity *in vivo*.

To measure the divalent cation specificities of the enzyme, FPT and GST-PEP were treated with EDTA. EDTA-treated enzyme showed little catalytic activity compared with the enzyme which was not treated with EDTA (Fig. 6a). When the enzyme was not treated with EDTA, addition of $MgCl_2$ at a concentration above 1.0 mM gave almost full activity. But EDTA-treated enzyme was no longer activated by $MgCl_2$, even at 5.0 mM. The activity of the EDTA-treated enzyme was restored when 20 μM $ZnCl_2$ was present together with 10 mM $MgCl_2$ (Fig. 6b). The EDTA-treated enzyme was not activated by addition of $ZnCl_2$ in the absence of $MgCl_2$ (Fig. 6c). In the presence of a saturating concentration of $MgCl_2$ (10 mM), maximal activation was observed at a $ZnCl_2$ concentration of 20 μM, even though 16 μM EDTA was present in the assay. But at the higher concentration of $ZnCl_2$, inhibition was observed. At a subsaturating concentration of $MgCl_2$ (5 mM), the addition of $ZnCl_2$ had a lesser effect. Consi-

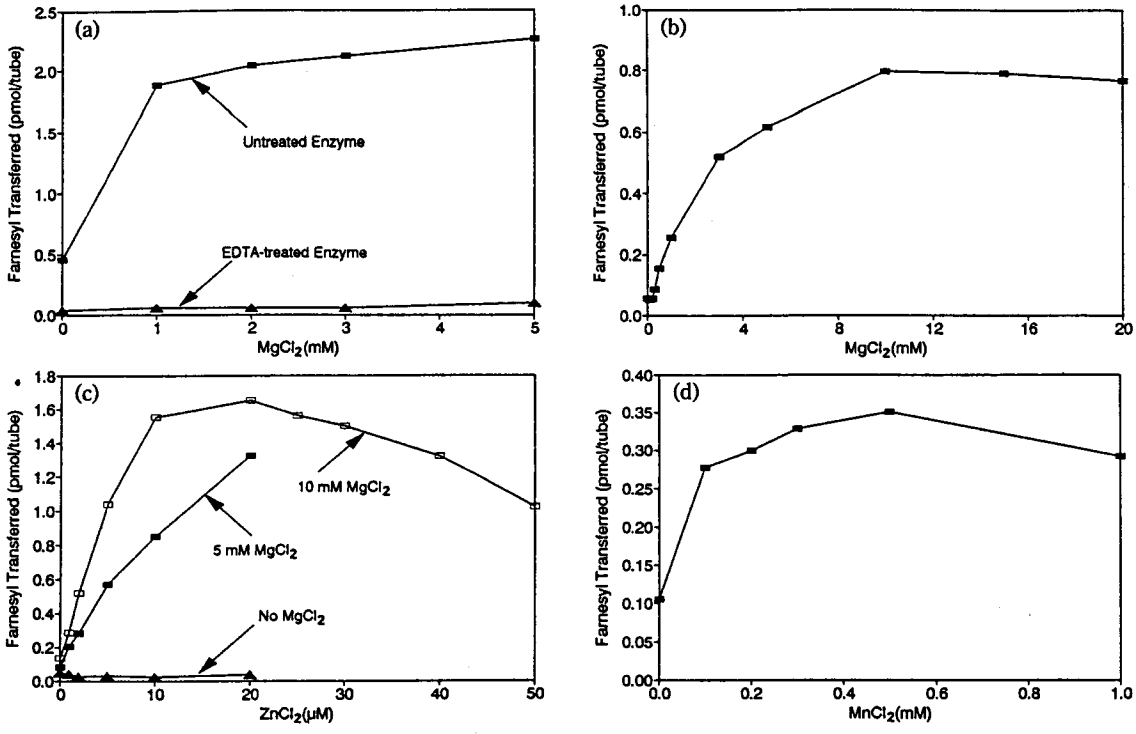

Fig. 6. Divalent cation specificities of FPT. (a) Lack of activation by Mg^{2+} of FPT activity of EDTA-treated enzyme. Reactions were carried out in the absence of $ZnCl_2$. (b) Mg^{2+} requirement for FPT activity. Reactions were carried out in the presence of 20 μM $ZnCl_2$. (c) Combined Zn^{2+} and Mg^{2+} requirement for FPT activity. (d) Mn^{2+} requirement for FPT activity. Reactions were carried out in the absence of $MgCl_2$. All reaction mixtures contained 16 μM EDTA.

dered together, the data of Fig. 6a-6c indicates that the EDTA-treated FPT requires both Mg^{2+} and Zn^{2+} for catalytic activity (Reiss *et al.*, 1992). Fig. 6d shows that $MnCl_2$ can substitute for $MgCl_2$ in FPT reaction (Manne *et al.*, 1990). In the case of $MnCl_2$, the optimal concentration was 0.5 mM, whereas with $MgCl_2$, optimal activity was observed at 10 mM. From these results, it is concluded that the FPT is a Zinc-metalloenzyme which has a tightly bound Zn^{2+}that cannot be removed easily, and needs Mg^{2+} for catalytic activity.

Acknowledgement

This research was supported by a grant from the ministry of education (1994).

References

Baik, Y. J. (1994) Doctor's Thesis, Dept. of Chem., Seoul Nat'l Univ., Seoul, Korea.

Casey, P. J. (1992) *J. Lipid Res.* **33**, 1731.

Chen, W.-J., Andres, D. A., Goldstein, J. L., Russell, D. W. and Brown, M. S. (1991) *Cell* **66**, 327.

Clarke, S. (1992) *Annu. Rev. Biochem.* **61**, 355.

CPMB (Current Protocols in Molecular Biology) (1987) John Wiley & Sons.

Gibbs, J. B. (1991) *Cell* **65**, 1.

He, B., Chen, P., Chen, S.-Y., Vancura, K. L., Michaelis, S. and Powers, S. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 11373.

Kinsella, B. T. and Maltese, W. A. (1991) *J. Biol. Chem.* **266**, 8540.

Laemmli, U. K. (1970) *Nature* **227**, 680.

Manne, V., Roberts, D., Tobin, A., O'Rourke, E., Virgilio, M. D., Meyers, C., Ahmed, N., Kurz, B., Resh, M., Kung, H.-F. and Barbacid, M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7541.

Moomaw, J. F. and Casey, P. J. (1992) *J. Biol. Chem.* **267**, 17438.

Moores, S. L., Schaber, M. D., Mosser, S. D., Rands, E., O' Hara, M. B., Garsky, V. M., Marshall, M. S., Pompliano, D. L. and Gibbs, J. B. (1991) *J. Biol. Chem.* **266**, 14603.

Pompliano, D. L., Rands, E., Schaber, M. D., Mosser, S. D., Anthony, N. J. and Gibbs, J. B. (1992) *Biochemistry* **31**, 3800.

Reiss, Y., Goldstein, J. L., Seabra, M. C., Casey, P. J. and Brown, M. S. (1990) *Cell* **62**, 81.

Reiss, Y., Stradley, S. J., Gierasch, L. M., Brown, M. S. and Goldstein, J. L. (1991a) *Proc. Natl. Acad. Sci. USA* **88**, 732.

Reiss, Y., Seabra, M. C., Armstrong, S. A., Slaughter, C. A., Goldstein, J. L. and Brown, M. S. (1991b) *J. Biol. Chem.* **266**, 10672.

Reiss, Y., Brown, M. S. and Goldstein, J. L. (1992) *J. Biol. Chem.* **267**, 6403.

Schaber, M. D., O'Hara, M. B., Garsky, V. M., Mosser, S. D., Bergstrom, J. D., Moores, S. L., Marshall, M. S., Friedman, P. A., Dixon, R. A. F. and Gibbs, J. B. (1990) *J. Biol. Chem.* **265**, 14701.

Seabra, M. C., Reiss, Y., Casey, P. J., Brown, M. S. and Goldstein, J. L. (1991) *Cell* **65**, 429