Substitution of Cadmium for Zinc in Farnesyl:Protein Transferase Alters Its Substrate Specificity†

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ABSTRACT: Ras proteins are mutationally activated in a variety of human cancers. Since farnesylation of Ras proteins is required for expression of their oncogenic potential, the enzyme responsible for this reaction, farnesyl:protein transferase (FPT), has become a major target for anticancer drug development. FPT is a zinc metalloenzyme, and the zinc is essential for its catalytic activity. To begin to elucidate the role of zinc in catalysis, we initiated metal substitution studies. Of all metals tested, only cadmium was able to functionally substitute for zinc, reconstituting enzymatic activity with native substrates (H-Ras and farnesyl diphosphate) to about 50% of that of the zinc-containing enzyme. Several important differences were observed between cadmium-substituted FPT (Cd-FPT) and zinc-containing FPT (Zn-FPT). Cd-FPT not only uses H-ras with its native CaaX motif (Ras-CVLS) as a substrate but also can farnesylate H-ras in which the CaaX motif is altered to contain a C-terminal leucine residue (Ras-CVLL). Similarly, Cd-FPT can farnesylate leucine-terminated peptides. Leucine-terminated proteins and peptides are usually substrates for the related enzyme geranylgeranyl:protein transferase type I. Farnesylation of Ras-CVLS and Ras-CVLL by Cd-FPT exhibited similar sensitivity to the FPT inhibitor SCH 44342 and to the peptide inhibitor CAIM. However, unlike Zn-FPT, Cd-FPT is also potently inhibited by the leucine-terminated peptide CAIL. These results indicate that the metal ion content of FPT strongly influences its protein substrate specificity.

Farnesyl:protein transferase (FPT)[1] transfers a farnesyl group from its prenyl donor farnesyl diphosphate (FPP) to the cysteine residue of substrate proteins containing a C-terminal CaaX motif in which X is methionine, serine, or glutamine (Reiss et al., 1990, 1991b). Known substrates of FPT include the H-, K-, and N-Ras proteins, nuclear lamins A and B, the γ subunit of the retinal trimeric G protein transducin, rhodopsin kinase, the α subunit of retinal cGMP phosphodiesterase, and a peroxisomal protein PxF (Clarke, 1992; Schafer & Rine, 1992; Glomset & Farnsworth, 1994; Zhang & Casey, 1996a; Casey & Seabra, 1996). Short peptides encompassing the CaaX motif of these substrates are also recognized by the enzyme (Reiss et al., 1990, 1991b). Native FPT has been purified from rat and bovine brain cytosol and is a heterodimeric protein of ~95 kDa containing two subunits designated as α (48 kDa) and β (45 kDa) (Reiss et al., 1990; Kohl et al., 1991). cDNA clones for both subunits have been isolated from rat, bovine, and human cDNA libraries (Chen et al., 1991a,b; Omer et al., 1993).

FPT is a zinc metalloenzyme, containing 1 mol of zinc per mole of enzyme (Moomaw & Casey, 1992; Reiss et al., 1992; Chen et al., 1993). The properties of FPT are similar to those of a related enzyme, geranylgeranyl:protein transferase type I (GGPT I). GGPT I transfers a geranylgeranyl group from the prenyl donor geranylgeranyl diphosphate (GGPP) to the cysteine residue of substrate proteins containing a C-terminal CaaX motif in which X is leucine (Casey et al., 1991; Yokoyama et al., 1991). The X residue of the CaaX motif is the primary determinant for recognition of a particular substrate protein by either FPT or GGPT I (Casey et al., 1991; Yokoyama et al., 1991; Moores et al., 1991). FPT and GGPT I share a common α subunit but contain distinct β subunits (Seabra et al., 1991; Zhang et al., 1994a; Mayer et al., 1992). Like FPT, GGPT I utilizes short peptide substrates and requires zinc for its activity (Moomaw & Casey, 1992; Yokoyama et al., 1993). A second geranylgeranyl:protein transferase (GGPT II) differs substantially from FPT. GGPT II transfers geranylgeranyl groups to both cysteine residues of substrate proteins containing a C-terminal CC or CxC motif. Most GGPT II substrates identified to date belong to the Rab family of small G proteins (Seabra et al., 1992; Horiuchi et al., 1991). In contrast to FPT and GGPT I, GGPT II cannot use short peptides as substrates (Wilson & Maltese, 1993; Beranger et al., 1994).

The zinc atom is an integral component of FPT, and its removal by prolonged dialysis against chelating agents completely inactivates the enzyme (Moomaw & Casey, 1992; Reiss et al., 1992). This metal-depleted FPT (designated as apo-FPT) retains high-affinity binding of the isoprenoid substrate FPP, but it can no longer be cross-linked to protein

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[1] Abbreviations: FPT, farnesyl:protein transferase; Cd-FPT, cadmium-reconstituted farnesyl:protein transferase; Zn-FPT, zinc-reconstituted farnesyl:protein transferase; FPP, farnesyl diphosphate; GGPT-I, geranylgeranyl:protein transferase type I; GGPT-II, geranylgeranyl:protein transferase type II; GGPP, geranylgeranyl diphosphate; Ras-CVLS, Ha-Ras protein terminating in Cys-Val-Leu-Ser; Ras-CVLL, Ha-Ras protein terminating in Cys-Val-Leu-Leu.

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substrates, indicating that the zinc is required for protein substrate binding (Reiss et al., 1991a, 1992). The activity of apo-FPT can be restored by simultaneous addition of both zinc and magnesium ions, but neither zinc nor magnesium alone restores activity (Moomaw & Casey, 1992; Reiss et al., 1992). Since the magnesium requirement for FPT activity is in the millimolar range, this metal is not considered to be an integral component of the enzyme.

Ras proteins are one class of FPT substrate of particular interest. These proteins are GTP-binding proteins that play crucial roles in cell growth and development. Mutations in Ras that result in its constitutive activation are associated with a broad range of human cancers (Barbacid, 1987; Lowy & Willumsen, 1993). Farnesylation of Ras has been shown to be required for oncogenic forms of the protein to transform cells, indicating that controlling farnesylation is a viable route to regulating Ras function (Casey et al., 1989; Schafer et al., 1989; Hancock et al., 1989). Numerous inhibitors have been developed for FPT (Gibbs et al., 1994; Zhang & Casey, 1996a). Both peptidomimetic and tricyclic inhibitors of FPT have been demonstrated to inhibit Ras farnesylation in cell culture and reverse cellular transformation induced by oncogenic Ras (James et al., 1993; Kohl et al., 1993; Bishop et al., 1995). Furthermore, such inhibitors can block Ras-dependent tumor growth in nude mice (Kohl, 1994).

In order to learn more about the role of zinc in catalysis by FPT, we have explored the ability of other metal ions to substitute for zinc. We show here that Cd^{2+} can reconstitute the activity of apo-FPT. Moreover, Cd^{2+}-substituted FPT not only prenylates its native substrate, Ras-CVLS, but also prenylates a standard substrate of GGPT-I, that being Ras-CVLL. These results indicate that the metal atom in FPT is important in enzyme interactions with protein substrates.

EXPERIMENTAL PROCEDURES

Materials. The FPT used in this study was the recombinant rat enzyme purified from the baculovirus/Sf9 cell expression system (Chen et al., 1993). Apo-FPT was prepared as described previously (Moomaw & Casey, 1992). H-Ras-CVLS and H-Ras-CVLL (an H-Ras protein with a C-terminal leucine-for-serine substitution) were purified from bacterial expression systems and dialyzed to remove potential contaminating metals as previously described (Zhang & Casey, 1996b). Protein concentrations were routinely determined using the Bradford method with a commercial dye preparation (Bio-Rad) using bovine serum albumin as the standard. Cadmium chloride (99.99%), mercury chloride (99.999%), zinc chloride (99.999%), cobalt chloride (99.999%), and nickel chloride (99.999%) were obtained from Aldrich. Ultrapure tris(hydroxymethyl)aminomethane, EDTA, magnesium chloride, manganese(II) chloride tetrahydrate, and potassium chloride were obtained from Fluka. [1-^{3}H]-Farnesyl diphosphate (22.5 Ci/mmol) and [1-^{3}H]geranylgeranyl diphosphate (19.3 Ci/mmol) were obtained from DuPont NEN. The biotinylated peptide KKSKTKCVIL was obtained from Analytical Biotechnology Services (Boston, MA). All water used in this study had a resistivity of ~18 MΩ/cm.

Reconstitution of Apo-FPT. Apo-FPT [1.1 mg/mL in 50 mM Tris-HCl (pH 7.8), 1 mM DTT, and 100 μM EDTA] was diluted 1:1 with 166 μM solutions of various metal chlorides ($CdCl_2$, $ZnCl_2$, $HgCl_2$, $MnCl_2$, $NiCl_2$, and $CoCl_2$). The concentration of free metal in the resultant reconstitution reactions was 33 μM. For some experiments, cadmium-substituted FPT was also prepared by reconstitution with $CdCl_2$ at concentrations up to 1 mM. The reconstitutions were carried out at 25 °C for 5 min. The reconstituted enzymes were then diluted in 50 mM Tris-HCl (pH 7.8), 20 mM KCl, 1 mM DTT, and 50 μM EDTA and assayed for FPT activity.

Determination of FPT Activity by Product Precipitation. FPT activity using protein substrates was determined by the method described previously involving acid precipitation of the prenylated product except that $ZnCl_2$ was omitted from the reaction mixture unless otherwise noted (Chen et al., 1993). The standard reaction mixture contained 50 mM Tris-HCl (pH 7.8), 1 mM DTT, 20 mM KCl, 3 μM Ras-CVLS, 0.5 μM [^{3}H]FPP (22.5 Ci/mmol), 5 mM $MgCl_2$, and 75 ng of purified recombinant FPT in a final volume of 50 μL. Assays were conducted at 37 °C for 15 min. For kinetic studies, the reaction mixture contained the following components in 2 mL: 50 mM Tris-HCl (pH 7.8), 1 mM DTT, 20 mM KCl, 0.4−3.0 μM Ras-CVLS or Ras-CVLL, 5 mM $MgCl_2$, and 0.05 μM [^{3}H]FPP (22.5 Ci/mmol). After pre-equilibration of the assay mixture at 30 °C, the reaction was initiated by addition of 50 ng of enzyme. At timed intervals, aliquots of 200 μL were withdrawn and reactions were terminated by addition of 0.5 mL of 6% SDS. Product was precipitated by addition of 0.5 mL of 30% TCA and processed by filtration through glass fiber filters as described (Zhang et al., 1994b). Reactions were never allowed to proceed to more than 10% completion based on the limiting substrate.

Determination of FPT Activity by Scintillation Proximity Assay. FPT activity utilizing peptide substrates was determined by measuring the transfer of [^{3}H]farnesyl from [^{3}H]-FPP to a biotinylated substrate peptide. The resultant prenylated peptide was captured by streptavidin-coated scintillation proximity assay (SPA) beads (Amersham). The standard assay mixture contained 50 mM Tris-HCl (pH 7.8), 1 mM DTT, 20 mM KCl, 300 nM biotinylated peptide, 200 nM [^{3}H]FPP (22.5 Ci/mmol), and 75 ng of purified recombinant FPT in a final volume of 50 μL. Assays were conducted at 25 °C for 20 min. SPA beads (50 mg) were first suspended in 2.5 mL of PBS and then diluted (1:15) in stop buffer [250 mM EDTA (pH 7.5) and 0.5% BSA]. Diluted SPA beads (0.2 mg in 150 μL) were added to terminate the reaction, and the resulting mixture was allowed to equilibrate at room temperature prior to quantitation by liquid scintillation spectroscopy.

Metal Chelation Experiments. Apo-FPT (2.2 μg) was first reconstituted with zinc or cadmium by incubating with the 2 mM appropriate ion. The reconstituted enzyme was then diluted 36-fold into 50 mM Tris-HCl (pH 7.8), 20 mM KCl, and 25 mM EDTA at 4 °C. At the indicated times, aliquots containing 75 ng of enzyme were withdrawn and assayed for FPT activity by the product precipitation method described above.

RESULTS

Reconstitution of Apo-FPT. To begin to study the functional role of zinc in FPT catalysis, we examined the ability of different divalent metal ions to restore activity to apo-FPT. Following reconstitution with various metal ions, enzymatic activity was measured in the presence of 5 mM Mg^{2+} using Ras-CVLS and FPP as substrates. Consistent

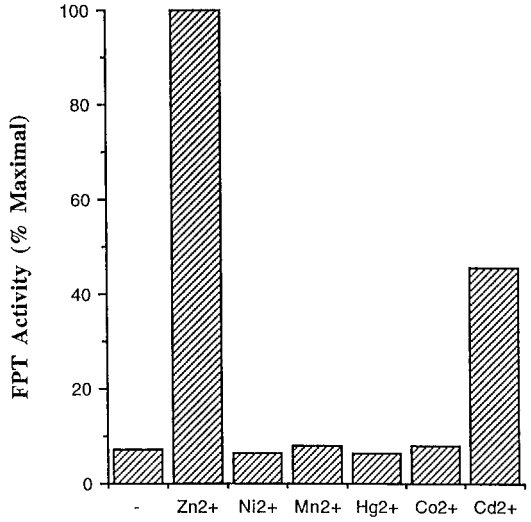

FIGURE 1: Reconstitution of apo-FPT by divalent metal ions. Apo-FPT was reconstituted in the presence of different metal chloride solutions ($ZnCl_2$, $NiCl_2$, $MnCl_2$, $HgCl_2$, $CoCl_2$, and $CdCl_2$) as described in Experimental Procedures. The reconstituted enzyme was then diluted and assayed in the presence of 5 mM Mg^{2+} using FPP and Ras-CVLS as substrates. Activities were reported relative to that of Zn-FPT (designated as 100%). The negative control is the activity of apo-FPT.

with previous results, Zn^{2+} fully restores the activity of apo-FPT (Moomaw & Casey, 1992; Reiss et al., 1992) (Figure 1). Cd^{2+} can also restore activity to apo-FPT, whereas Ni^{2+}, Mn^{2+}, Hg^{2+}, and Co^{2+} cannot. The specific activity of Cd^{2+}-reconstituted FPT (Cd-FPT) was about 50% of that of Zn-FPT using Ras-CVLS and FPP as substrates.

Cd-FPT Has Altered Substrate Specificity. The substrate specificity of Cd-FPT was characterized using various combinations of isoprenyl diphosphate substrates (FPP or GGPP) and protein substrates (H-Ras-CVLS or H-Ras-CVLL). Cd-FPT displayed quite high activity when FPP and Ras-CVLS were used as substrates. The ability of Cd-FPT to transfer the geranylgeranyl group from GGPP to either Ras-CVLS or Ras-CVLL is very low (Figure 2A). These properties are similar to those of Zn-FPT. However, in contrast to Zn-FPT, Cd-FPT exhibits substantial activity using FPP and Ras-CVLL as substrates; this activity is at least 14-fold greater than that of Zn-FPT for the same reaction. Therefore, the protein substrate specificity of Cd-FPT is substantially relaxed compared to that of Zn-FPT.

The relative affinities of the various reconstituted forms of FPT for Ras-CVLS and Ras-CVLL substrates were compared. The K_m of Zn-FPT for Ras-CVLS determined in this study is 0.57 μM, while that of Cd-FPT is 0.80 μM, suggesting that Cd-FPT interacts with this substrate with affinity essentially identical to that of the zinc-containing enzyme (Figure 2B). For Ras-CVLL, Cd-FPT exhibits a K_m value of 7.7 μM, while Zn-FPT displays only weak activity with this substrate at concentrations up to 10 μM. This indicates much more efficient utilization of the leucine-terminated protein by the cadmium-reconstituted enzyme. Therefore, cadmium substitution dramatically increases the affinity of FPT for Ras-CVLL, while not affecting its affinity for Ras-CVLS.

The altered substrate specificity of Cd-FPT was confirmed using a peptide substrate. KKSKTKCVIL is a peptide corresponding to the C-terminus of K-Ras except that Leu is substituted for Met at the C-terminal residue. Biotinylated KKSKTKCVIL was tested as a substrate for Cd-FPT and

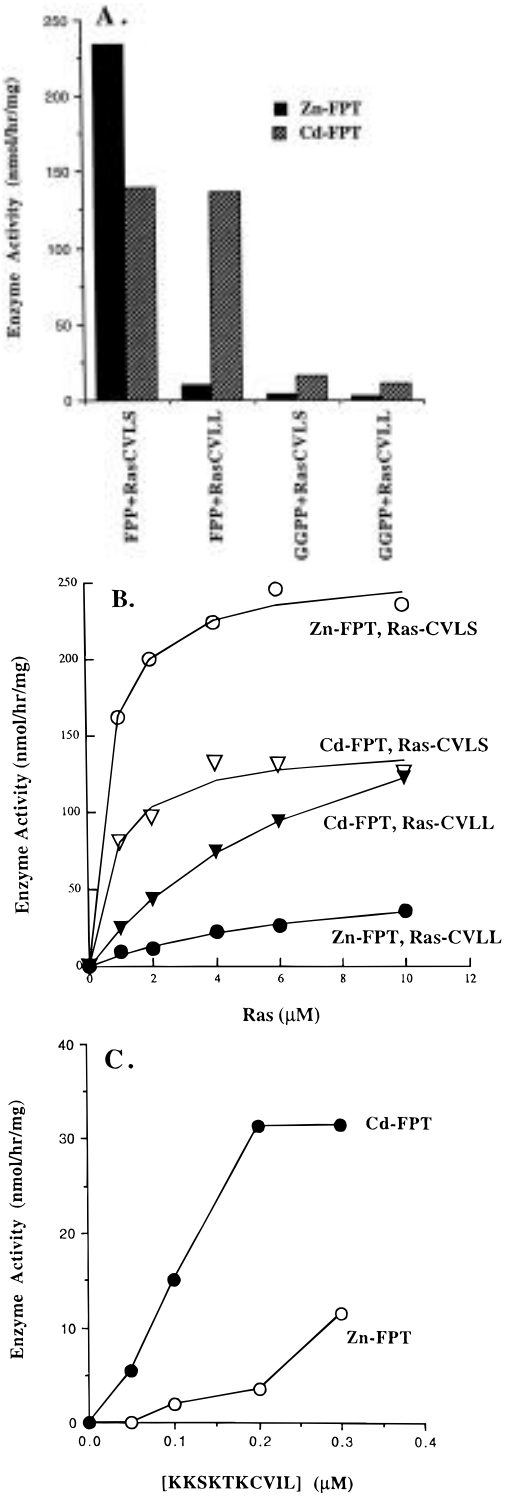

FIGURE 2: Substrate specificity of Zn-FPT and Cd-FPT. (A) Analysis using various isoprenoid and protein substrate combinations. The indicated substrate combinations were used to assay the activity of Zn-FPT and Cd-FPT. Assay mixtures were identical to the standard reaction mixture, except that the indicated substrates were used. The concentration of isoprenoid substrate (FPP or GGPP) was 0.5 μM, while the concentration of protein substrates (Ras-CVLS or Ras-CVLL) was 3 μM. Assays were conducted in the presence of 5 mM Mg^{2+}. (B) Protein substrate saturation curves. The assay method was the same as in panel A except the concentrations of Ras-CVLS and Ras-CVLL were varied as indicated. (C) Analysis using peptide substrates. The biotinylated peptide KKSKTKCVIL and FPP were used as substrates to assay Cd-FPT and Zn-FPT. Assay conditions were as described in Experimental Procedures.

Zn-FPT using the scintillation proximity method. As expected, this peptide is a poor substrate for Zn-FPT;

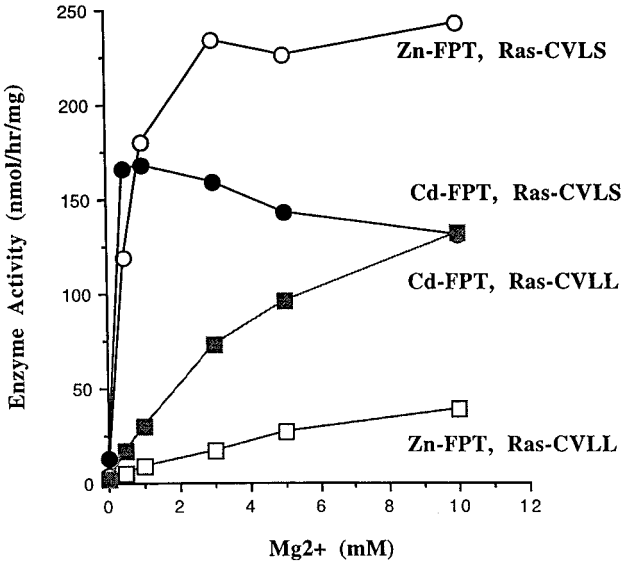

FIGURE 3: Mg^{2+} dependence of Zn-FPT and Cd-FPT. Assay methods were the same as employed in Figure 2A, except that the concentration of Mg^{2+} was varied as indicated.

however, it serves as an efficient substrate for Cd-FPT (Figure 2C). This result is consistent with results obtained using protein substrates and indicates that cadmium substitution changes the specificity of FPT for peptides, as well as for protein substrates.

Mg^{2+} Dependence of Zn-FPT and Cd-FPT. Native FPT requires both zinc and magnesium for its activity. After establishing that cadmium substitution alters the substrate specificity of FPT, we next determined whether Cd-FPT has the same requirement for Mg^{2+} as Zn-FPT. Using H-Ras-CVLS as a substrate, Zn-FPT required 3 mM Mg^{2+} for maximal activity, while this was reduced to approximately 0.5 mM Mg^{2+} for Cd-FPT (Figure 3). Both forms of the enzyme require higher Mg^{2+} concentrations when Ras-CVLL serves as the prenyl acceptor, with saturation of activity not achieved at 10 mM $MgCl_2$. In the presence of 10 mM Mg^{2+}, the activity of Zn-FPT is about 25% of the Cd-FPT activity. Therefore, both Cd-FPT and Zn-FPT require Mg^{2+} for their activity, but the Mg^{2+} requirement for Cd-FPT is reduced.

Sensitivity of Cd-FPT to FPT Inhibitors. We also compared the sensitivity of Cd-FPT and Zn-FPT to three inhibitors. The tetrapeptide CAIM, which corresponds to the C-terminus of lamin B, is a substrate-active inhibitor of Zn-FPT (Reiss et al., 1991b; Goldstein et al., 1991). The tetrapeptide CAIL is similar to CAIM except that the C-terminal residue is changed from Met to Leu. This peptide is a substrate-active inhibitor of GGPT-I (Moores et al., 1991). SCH 44342 is a nonpeptidic tricyclic inhibitor that exhibits specificity toward FPT (Bishop et al., 1995). CAIM inhibits farnesylation of Ras-CVLS and Ras-CVLL by Cd-FPT with IC_{50} values of 0.4 and 0.35 μM, respectively (Table 1). This is identical to the potency of this peptide for inhibiting the farnesylation of Ras-CVLS by Zn-FPT (0.4 μM). SCH 44342 inhibits farnesylation of Ras-CVLS and Ras-CVLL by Cd-FPT with IC_{50} values of 1.8 and 0.9 μM, respectively. Again, this is similar to the potency of this compound for inhibition of Zn-FPT (0.8 μM under the assay conditions employed here) (Table 1). Thus, CAIM and SCH 44342 have similar potency for both reactions catalyzed by Cd-FPT; moreover, their potency is similar to that for Zn-FPT. Inhibition of Cd-FPT by the peptide CAIL was also similar using either Ras-CVLL (IC_{50} = 2.8 μM) or Ras-CVLS (IC_{50} = 3.0 μM) as substrate. However, CAIL is a much poorer inhibitor of Zn-FPT (IC_{50} of approximately 30 μM) (Figure 4). Therefore, cadmium substitution increased the affinity of FPT for the leucine-terminated peptide but does not affect its affinity for the methionine-terminated peptide, consistent with results obtained with protein and peptide substrates.

Table 1: IC_{50} Values for Inhibitors versus Zn-FPT and Cd-FPT[a]

inhibitor	Cd-FPT Ras-CVLS	Cd-FPT Ras-CVLL	Zn-FPT Ras-CVLS
CAIM	0.4 μM	0.35 μM	0.4 μM
SCH 44342	1.8 μM	0.9 μM	0.8 μM
CAIL	3.0 μM	2.8 μM	30.0 μM

[a] Reactions were carried out as described in Experimental Procedures. Reaction mixtures contained varying concentrations of competitor peptide or SCH 44342. Activities were calculated relative to vehicle (DMSO) control (100%), and IC_{50} values were determined from plots of activity versus inhibitor concentration.

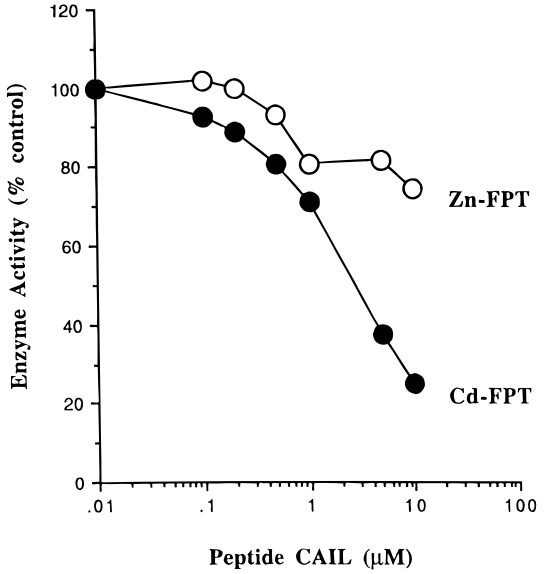

FIGURE 4: Inhibition of Cd-FPT and Zn-FPT by CAIL. The ability of the peptide inhibitor CAIL to inhibit the cadmium and zinc-reconstituted forms of FPT was compared. Standard reaction conditions were employed, and Ras-CVLS and FPP served as substrates. The concentration of CAIL was varied, and percent inhibition was calculated relative to control reactions in the absence of inhibitor.

Kinetic Analysis of Inhibition of Cd-FPT by a Dead-End Substrate. Since Cd-FPT uses both Ras-CVLS and Ras-CVLL as substrates, it was important to determine whether these proteins bind to the same site on the enzyme. Kinetic studies with a dead-end inhibitor of Cd-FPT can be used to evaluate this. The tetrapeptide CVFM was previously shown to be a dead-end substrate for Zn-FPT, and we confirmed that this is also the case for Cd-FPT (data not shown). The inhibitory properties of CVFM with respect to farnesylation of both Ras-CVLS and Ras-CVLL by Cd-FPT were examined. Analysis of the data via standard double-reciprocal plots revealed that CVFM is a competitive inhibitor with respect to modification of both protein substrates (Figure 5). These results suggest that, as expected, the binding sites on Cd-FPT for Ras-CVLS and Ras-CVLL overlap.

Stability of Cd-FPT and Zn-FPT. In order to compare the stability of cadmium-substituted FPT with that of Zn-FPT, both metal-reconstituted forms of the enzyme were incubated with 25 mM EDTA at 0 °C. The enzymatic

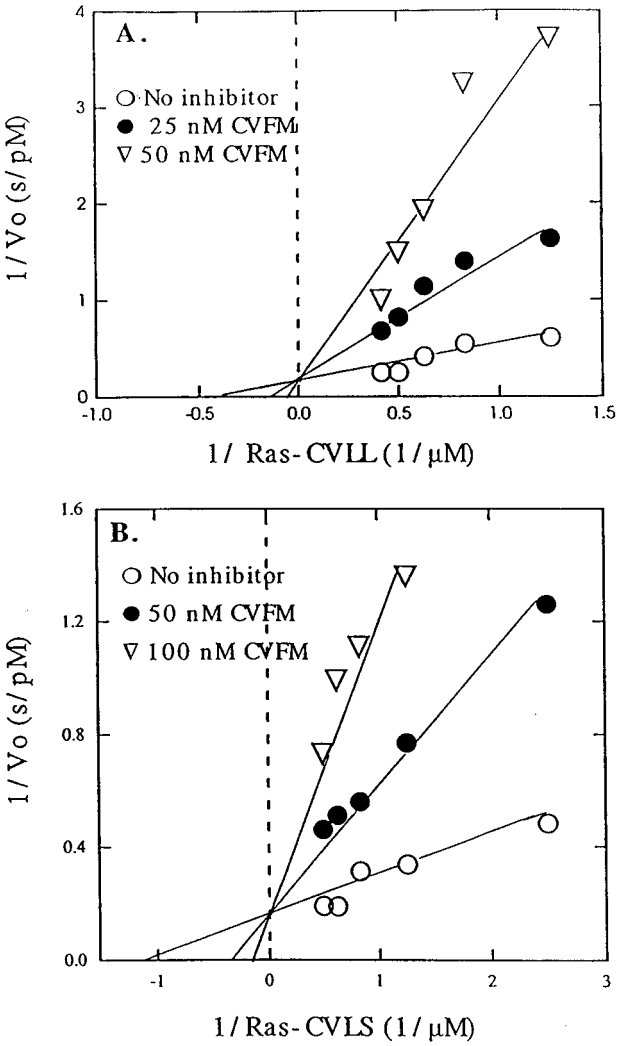

FIGURE 5: Kinetic analysis of Cd-FPT. (A) Competition with respect to Ras-CVLL. The concentration of FPP was fixed at 50 nM, and the initial velocities were determined in the presence of either no inhibitor (○), 25 nM CVFM (●), or 50 nM CVFM (▽). The data are plotted as double-reciprocal plots of velocity versus substrate concentration. (B) Competition with respect to Ras-CVLS. The concentration of FPP was fixed at 50 nM, and the initial velocities were determined in the presence of either no inhibitor (○), 50 nM CVFM (●), or 100 nM CVFM (▽).

activities were assayed (using FPP and Ras-CVLS as substrates) following different incubation times with EDTA. The activities of both Cd-FPT and Zn-FPT decrease with incubation time, reflecting chelation of the metal ions from the enzyme (Figure 6). The rate of activity loss for Zn-FPT ($k = 0.012$ min^{-1}) is 2-fold faster than that for Cd-FPT ($k = 0.006$ min^{-1}), suggesting that cadmium is somewhat more tightly bound to the enzyme than zinc.

DISCUSSION

Among all the metal ions tested, only Cd^{2+} could restore activity to metal-depleted FPT. Ni^{2+}, Mn^{2+}, Hg^{2+}, or Co^{2+} was unable to restore activity. Both cadmium and zinc are group IIB metals (Weast et al., 1987), indicating that the coordination preference of metal ions is important for their ability to reconstitute enzyme activity. Cadmium has a larger ionic radius and binds to the enzyme with a higher affinity than zinc. Although mercury is located in the same group as cadmium and zinc, the ionic radius of Hg^{2+} is substantially larger than that of Cd^{2+}, and thus, it probably cannot fit into the metal binding site. It is quite likely that Cd^{2+} occupies

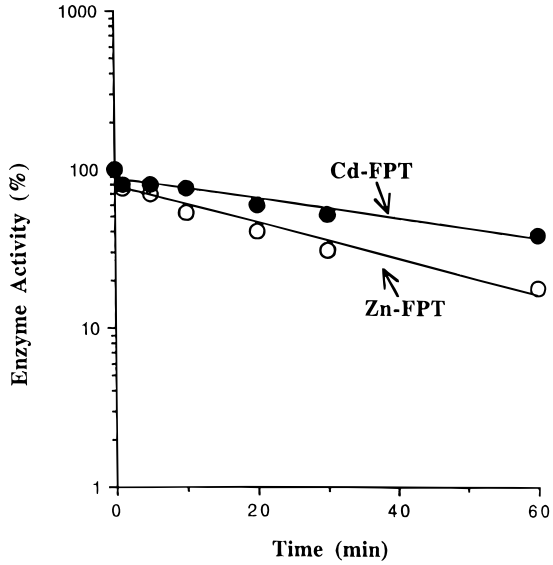

FIGURE 6: Stability of Zn-FPT and Cd-FPT. Reconstituted Zn-FPT or Cd-FPT was incubated with 25 mM EDTA at 4 °C. At the indicated times, aliquots were withdrawn and assayed using FPP and Ras-CVLS as substrates. Activities were reported relative to that of enzyme not incubated with EDTA (designated as 100%). The assays were conducted as described in Experimental Procedures.

the same site as Zn^{2+} in the enzyme for the following reasons. First, Cd-FPT retains activity with the standard Zn-FPT substrates, Ras-CVLS and FPP, indicating that the substrate binding sites are not disrupted. Second, like Zn-FPT, Cd-FPT also requires Mg^{2+} for its activity, although the amount of Mg^{2+} needed for maximal activity is somewhat reduced. Third, known FPT inhibitors, CAIM and SCH 44342, have similar potencies against both forms of the enzyme.

FPT uses protein substrates containing CaaX motifs that terminate in Ser, Met, and Gln but shows poor activity with substrates that terminate in Leu (Clarke, 1992; Zhang & Casey, 1996). We have found that Cd-substituted FPT differs from Zn-FPT in several important respects. Cd-FPT not only uses the standard FPT substrate Ras-CVLS but also can farnesylate leucine-terminated protein substrates (e.g. Ras-CVLL) with comparable efficiency, suggesting that the binding site for the protein substrates is more flexible in Cd-FPT than in Zn-FPT. This assessment is supported by experiments using peptide substrates and inhibitors. A peptide that serves as a substrate for GGPT-I (KKSKT-KCVIL) is a poor substrate of Zn-FPT; however, it is a relatively efficient substrate for Cd-FPT. Furthermore, the peptide CAIL is a much more effective inhibitor of Cd-FPT than of Zn-FPT.

There are two ways in which cadmium could alter the protein substrate binding site of FPT. One is that the metal may directly coordinate protein substrates, probably through the sulfhydryl group of the CaaX motif. In this case, cadmium substitution may directly increase the affinity for Ras-CVLL in the ternary E·FPP·Ras complex. Another possibility is that the metal influences the conformation of the protein substrate binding site and that Cd^{2+} alters the conformation of this binding site. Further experiments are needed to distinguish between these and other potential possibilities. Interestingly, Cd-FPT behaves in a manner similar to that of a mutant form of FPT which was obtained by random mutagenesis of the yeast *Saccharomyces cerevisiae* (Mitsuzawa et al., 1995). This altered form of FPT

contains a single point mutation (Ser-159 to Asn) in the β subunit. Ser159 Asn FPT has increased ability to farnesylate a GGPT-I substrate (GST-CIIL) but reduced ability to farnesylate its own substrate (GST-GIIS). Similar to cadmium substitution, this mutation may be located in the protein binding region or may affect the folding of the binding site.

Cadmium has previously been shown to be able to substitute for zinc in GGPT-I (Yokoyama et al., 1995; Zhang & Casey, 1996b). Cd-GGPT-I has altered isoprenoid substrate specificity, with an increased ability to utilize FPP as substrate (Zhang & Casey, 1996b). The protein substrate specificity of GGPT-I is not affected as dramatically by cadmium substitution as is that of FPT. Although it does have an increased ability to utilize the peptides CVFL and CVFM (normally dead-end substrates for GGPT-I and FPT, respectively) (Zhang & Casey, 1996b), it cannot use Ras-CVLS as a substrate. These data suggest that, in the case of GGPT-1, the metal substitution primarily alters the isoprenoid binding site. In contrast, Cd-FPT has altered protein substrate specificity and unchanged isoprenoid substrate specificity. These results suggest that the influence of the metal ion on substrate binding in FPT and GGPT-1 may differ somewhat. Cd-FPT may be useful as an intermediate model for mechanistic studies of these prenyltransferases.

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