Burkitt Lymphoma Daudi Cells Contain Two Distinct Farnesyltransferases with Different Divalent Cation Requirements†

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ABSTRACT: Farnesylation is a lipid posttranslational modification required for the biological function of several signaling proteins including the Ras oncoprotein where this modification is required for malignant transformation. Here we report the identification of two distinct farnesyltransferases (FTases) in Burkitt lymphoma Daudi cells. Separation of Daudi cell cytosolic fractions by ion exchange chromatography resulted in two peaks (FTases I and II) that, on gel filtration, show molecular masses of 90 000 and 250 000 Da, respectively. Immunoblotting experiments showed that FTase I is composed of an α/β-heterodimer of about 50 000 Da each. FTase II contained a β-subunit that is immunologically indistinguishable from the β-subunit of FTase I and the previously reported human and rat brain FTase but contained an α-subunit that reacted poorly with a rat brain anti-α-antibody. As in rat brain FTase, Daudi FTases I and II both required magnesium for enzymatic activity. However, their zinc requirements differed. In the absence of Zn^{2+}, FTase I had little activity (10%) whereas FTase II had 30% of its maximum activity (maximum activity obtained in the presence of Zn^{2+}). Furthermore, whereas both FTases I and II were potently inhibited by K_B-Ras C-terminal Cys-Val-Ile-Met tetrapeptide mimics, only FTase I but not FTase II required zinc for peptide binding and inhibition.

Posttranslational modifications of cysteine thiols by the lipids farnesyl and geranylgeranyl are required for proper membrane localization and biological activity of several cellular proteins (Cox & Der, 1992). To date, two geranylgeranyltransferases (GGTases I and II) and one farnesyltransferase (FTase)[1] have been identified as enzymes responsible for catalyzing these lipid modifications (Casey, 1992). Both FTase and GGTase I recognize and prenylate proteins that end at their carboxyl termini with the sequence CAAX, where C is cysteine, A is any aliphatic amino acid, and X is methionine, serine, glutamine, or cysteine but not leucine or isoleucine (FTase) or X is leucine or isoleucine (GGTase I). Prenylation of the cysteines is followed by proteolytic removal of the tripeptide AAX and methylation of the resulting S-prenylated cysteine carboxyl group (Hancock et al., 1989). GGTase II, on the other hand, geranylgeranylates proteins that end at their carboxyl terminus in CXC, CC, or CCXX sequences, where X is any amino acid (Seabra et al., 1992; Farnsworth et al., 1994).

A variety of proteins has been shown to be prenylated, but the number of geranylgeranylated proteins exceeds that of farnesylated proteins by about a factor of 5 (Farnsworth et al., 1990; Casey, 1992). Interest in protein prenylation has recently intensified with the discovery that the Ras oncoproteins require farnesylation for their malignant transformation (Casey et al., 1989; Jackson et al., 1990). This has prompted many investigators to study the mechanism of farnesylation with the ultimate goal of designing agents that inhibit FTase and eventually tumor growth (Gibbs et al., 1994). We (Nigam et al., 1993; Qian et al., 1994a,b; Vogt et al., 1995) and others (Garcia et al., 1993; James et al., 1993; Graham et al., 1994) have recently designed Ras CAAX peptidomimetics that are potent inhibitors of FTase and Ras processing.

FTase has been purified to homogeneity from both bovine and rat brain (Reiss et al., 1990; Moores et al., 1991) and was shown to be a heterodimer composed of an α-subunit of 49 kDa and a β-subunit of 46 kDa (Reiss et al., 1991a). Cross-linking studies suggested that the β-subunit of FTase is involved in the binding of both substrates, p21 Ras (Reiss et al., 1992), and farnesylpyrophosphate (FPP) (Omer et al., 1993).

The α-subunit of FTase is shared with the closely related enzyme GGTase I (Seabra et al., 1991), but their β-subunits are distinct. cDNAs from both α- and β-subunits have been cloned from rat brain (Chen et al., 1991a,b) and, more recently, from human placental cDNA libraries (Omer et al., 1993). The deduced amino acid sequence of human FTase was shown to be 93−95% homologous to its rat brain counterpart (Omer et al., 1993). Recombinant FTase has been overexpressed in Sf9 cells using a baculovirus system (Chen et al., 1993). Coexpression of both subunits was shown to be required for FTase activity (Chen et al., 1991a,b, 1993), and it has been suggested that neither subunit alone is stable in the absence of the other (Chen et al., 1991b).

Purified recombinant rat brain FTase was shown to contain zinc, and because removal of this cation (which could only

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[1] Abbreviations: FTase, farnesyltransferase; GGTase, geranylgeranyltransferase; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; TBS-T, Tris-buffered saline with 0.05% Tween 20; CAAX, tetrapeptides where C = cysteine, A = aliphatic amino acid, and X = methionine or serine; DTT, dithiothreitol; FPP, farnesylpyrophosphate; FBS, fetal bovine serum; PBS, phosphate-buffered saline.

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be achieved by extensive dialysis against EDTA) resulted in loss of enzymatic activity, FTase was assigned to be a Zn metalloenzyme (Reiss et al., 1991a, 1992; Chen et al., 1993). Substrate-binding experiments have further established the necessity of Zn^{2+} for the binding of p21 Ras to the β-subunit and of Mg^{2+} for the transfer of enzyme-bound FPP (Reiss et al., 1991a, 1992) to suitable acceptors. Addition of Zn^{2+} to EDTA-dialzyed FTase restored binding of the p21 Ras substrate and enzymatic activity (Reiss et al., 1992; Moomaw & Casey, 1992). An earlier model suggested that Zn^{2+} plays a key role in the conformation of the C-terminal CAAX tetrapeptide of Ras at the active site of FTase. It was proposed that the tetrapeptide takes up a β-turn conformation that would bring in close proximity the cysteine thiol and the methionine carboxylate to coordinate Zn^{2+} in a bidentate complex (James et al., 1993). However, we have recently designed CAAX peptidomimetics that are potent inhibitors of FTase but that cannot take up a β-turn conformation (Nigam et al., 1993; Qian et al., 1994a,b; Vogt et al., 1995). This suggested that a β-turn conformation, which may exist at the active site, is not required for potent FTase inhibition. Furthermore, the amino acid sequence of the β-subunit of rat brain FTase did not reveal any known metal-binding consensus sequences (Chen et al., 1991b). It is therefore not clear at this point whether zinc ions play a catalytic or structural role in FTase biochemistry.

The majority of work has been done on rat brain FTase, but FTase activities have also been identified in various other tissues (Manne et al., 1990; Moores et al., 1991; Ray & Lopez-Belmonte, 1992). To date, it is generally accepted that there is only one kind of FTase, even though there have been some observations of molecular heterogeneity between FTases found in different tissues (Manne et al., 1990; Ray & Lopez-Belmonte, 1992). Furthermore, few reports have addressed properties of FTase from human tumors (Nigam et al., 1993; Vogt et al., 1995). In this paper, we present evidence that Burkitt lymphoma Daudi cells contain two distinct FTases, which appear to differ in charge, molecular size, α-subunit, and zinc requirements.

MATERIALS AND METHODS

Purification of FTases. All purification steps were performed at 4 °C. Human Burkitt lymphoma Daudi cells (ATCC, Rockville, MD) were grown in suspension in RPMI 1640 medium. NIH 3T3 (ATCC) and Calu I (ATCC) cells were grown in monolayers to 100% confluency in a humidified 10% CO_2 incubator at 37 °C in DMEM and McCoy's 5A modified medium, respectively. All media contained 10% fetal bovine serum (FBS) and 1% penicillin−streptomycin (GIBCO BRL). The cells were harvested and sonicated in 50 mM Tris (pH 7.5), 1 mM EDTA, 1 mM EGTA, 25 μg/mL leupeptin, and 1 mM phenylmethanesulfonyl fluoride. Homogenates were then spun at 12000*g*, and the resulting supernatant was further spun at 60000*g* (60S).

Cytosolic fractions from a male Sprague−Dawley rat brain and human tonsils taken from a healthy patient were prepared by washing the whole organs with ice-cold PBS followed by homogenizing in the above sonication buffer. Human lymphocytes were isolated from a Leukopack (Pittsburgh Central Blood Bank) and processed as described for the cultured cells. Rat brain FTase purified to homogeneity was a gift from Dr. P. Casey (Duke University, NC).

Mono-Q Chromatography. Postmicrosomal (60S) fractions (20 mg) were loaded onto a Mono-Q HR 5/5 column (Pharmacia Biotech), equilibrated in 50 mM Tris-Cl (pH 7.5), 20 μM $ZnCl_2$, 1 mM DTT, and 20 mM NaCl. Elution was carried out at 1 mL/min. The column was washed with 10 mL of the same buffer followed by a 15 mL linear gradient from 0.02 to 0.27 M NaCl followed by a second wash with 10 mL of buffer containing 0.27 M NaCl to elute FTase I. A 35 mL linear gradient from 0.27 to 1.02 M NaCl eluted FTase II in fractions containing 0.45 M NaCl; 1 mL fractions were collected and assayed for FTase activity (see below). Fractions containing FTase I and II activities were pooled separately, concentrated in Amicon CF25 Centriflo ultrafiltration cones to ca. 1 mL, and frozen at −70 °C until further use. Unless otherwise stated, experiments in this paper were performed with FTases partially purified by Mono-Q chromatography. For the preparation of metal-free enzyme, $ZnCl_2$ was omitted from the elution buffers.

Gel Filtration. A Superose 12 HR 12/30 column (Pharmacia) was equilibrated in 50 mM Tris-Cl (pH 7.5), 20 μM $ZnCl_2$, 1 mM DTT, and 200 mM NaCl to eliminate ionic interactions. The column was calibrated with known molecular mass markers as described in the legend to Figure 2. This calibration was repeated between runs of FTases; 0.8 mg of each Mono-Q-purified peak was applied to the column and chromatographed using the same buffer at 0.2 mL/min. Fractions (0.5 mL) were collected and assayed for FTase activity (see below).

Assay for FTase and GGTase I Activity. Assays for FTase activity were carried out as described previously (Nigam et al., 1993). Briefly, 20 μL of FTase was incubated in 50 mM Tris (pH 7.5), 50 μM $ZnCl_2$, 20 mM KCl, 3 mM $MgCl_2$, and 1 mM DTT. The reaction mixture was incubated at 37 °C for 30 min in the presence of 14 μM recombinant H-Ras-CVLS and 625 nM [^{3}H]FPP (Amersham; 16.3 Ci/mmol). The reaction was stopped, and the solution was passed through glass fiber filters to separate free and incorporated label.

For the determination of divalent metal ion requirements, FTases were rendered metal-free as described for GGTase I (Yokoyama et al., 1995). All buffers and solutions used in the preparation and characterization of metal-free enzyme were extracted with dithizone as described (Vallee & Galdes, 1984; Holmquist, 1988). Tris-Cl, potassium chloride, magnesium chloride, and EDTA were purchased from Sigma, and DTT was from Boehringer Mannheim. Zinc chloride (99.999%) was purchased from Aldrich and used without further treatment.

Plasticware used in the preparation and storage of FTases and solutions was soaked overnight in 10 mM EDTA, rinsed with Milli-Q water, and dried at 60 °C. Reactions were carried out in poly(propylene) tubes. Dialysis tubing was rendered metal-free by treatment with four changes of Milli-Q water at 70 °C and stored in water at 4 °C until used. FTases were dialyzed for 2 days at 4 °C against three changes of 100 vol of dithizone-treated Tris-Cl (pH 7.5), 0.1 M NaCl, 1 mM DTT, and 0.05 mM EDTA (buffer A).

Recombinant H-Ras-CVLS was prepared as described previously (Nigam et al., 1993) from bacteria obtained from Dr. Robert Crowl (Hoffman LaRoche Inc.) (Lacal et al., 1984) and dialyzed for 2 days against three changes of 100 vol of dithizone-treated buffer A containing 50 μM GDP. $MgCl_2$ and $ZnCl_2$ were omitted in the assay mixtures and

later added in appropriate concentrations as indicated in the figure legends. For inhibition studies, the peptidomimetics (Vogt et al., 1995) were premixed with FTase before adding the remainder of the reaction mixture.

GGTase I was assayed as described by Vogt et al. (1995). Briefly, fractions from the Mono-Q column were assayed for GGTase by determining their ability to transfer [^{3}H]-geranylgeranyl from [^{3}H]geranylgeranylpyrophosphate to p21 Ras-CVLL. Ras-CVLL protein was purified from bacteria expressing recombinant Ras-CVLL (Cox et al., 1992). These bacteria were kindly provided by Channing Der and Adrienne Cox (University of North Carolina, Chapel Hill, NC).

Western Blot Analysis. FTase samples at different stages of purification containing 3.0 U (3.0 pmol of FPP/h transferred to H-Ras-CVLS) of FTase activity were heated for 5 min in SDS−PAGE sample buffer, electrophoresed on 10% SDS−PAGE, and transferred onto nitrocellulose. The blots were blocked with 5% nonfat dry milk in TBS-T (pH 8.0) and probed with rabbit polyclonal antibodies against the α-subunit (C-19, 1 μg/mL) and the β-subunit (X-28, 0.1 μg/mL) of rat brain FTase (Santa Cruz Biotechnology, Santa Cruz, CA) in 5% nonfat dry milk in TBS-T. Positive antibody reactions were visualized using horseradish peroxidase-conjugated goat anti-rabbit IgG (Oncogene Science, Uniondale, NY) and an enhanced chemiluminescence detection sytem (ECL; Amersham). Blots were exposed to X-ray film for 30 s to 5 min.

RESULTS AND DISCUSSION

FTase is a cytosolic enzyme that catalyzes the transfer of farnesyl from the cholesterol biosynthesis intermediate farnesylpyrophosphate (FPP) to the cysteine thiol of C-terminal CAAX sequences of various proteins to form a thioether linkage (Casey et al., 1989; Reiss et al., 1991b). This lipid posttranslational modification allows these proteins to localize to various cellular membranes and function properly in their microenvironment. Although there are several proteins that are farnesylated, only one FTase has been reported. In this paper, we describe two FTases with distinct biochemical properties.

We first showed that Daudi cells have two FTase activities that can be separated on the basis of their overall charge. Postmicrosomal cytosolic fractions from Daudi cells were chromatographed on a Mono-Q anion exchange column as described in Materials and Methods. Figure 1A shows the elution profile after a NaCl gradient where two FTase enzymatic activities were well separated and eluted at 0.27 M NaCl (FTase I) and 0.45 M NaCl (FTase II). Analysis of Mono-Q fractions for GGTase I activity as described in Materials and Methods indicated that Daudi cells contain only one GGTase I that eluted prior to FTase I at 0.25 M NaCl (data not shown). Thus, neither FTase I nor FTase II have GGTase I activity associated with them. Figure 1B shows the Mono-Q elution profile of rat brain cytosolic fractions that were subjected to the identical purification procedure as for Daudi cells. In contrast to Daudi, rat brain contains mainly one FTase that eluted at the same relative position as Daudi FTase I (Figure 1A, B). This is consistent with previous reports that also showed rat brain to contain only one FTase (Reiss et al., 1990; Seabra et al., 1991).

In order to determine how common the presence of peak II is in other tissue sources, we have used Mono-Q

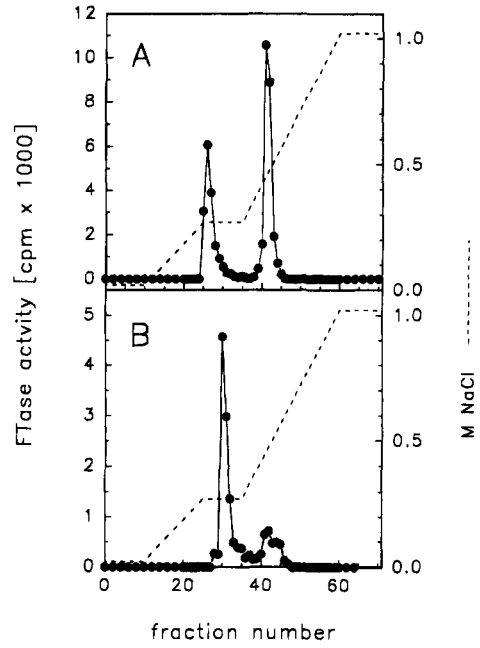

FIGURE 1: Mono-Q chromatography. Postmicrosomal cytosolic fractions from Burkitt lymphoma Daudi cells (A) or rat brain (B) were applied to a Mono-Q HR 5/5 column and eluted with a NaCl gradient at 1 mL/min; 1 mL fractions were collected and assayed for FTase activity as described under Materials and Methods. The two peaks of farnesyltransferase that eluted at 0.27 and 0.45 M NaCl, respectively, were termed "FTase I and FTase II". Data in panel A are representative of 10 independent experiments.

Table 1: Prevalence of FTases I and II in Different Cell Lines and Organ Tissues[a]

cell line/tissue	prevalence (%)[b]	
	FTase I	FTase II
Daudi	45	55
Calu I	50	50
NIH 3T3	50	50
human tonsils	70	30
human lymphocytes	85	15
rat brain	90	10

[a] Postmicrosomal cytosolic fractions from various cell lines and organ tissues were subjected to Mono-Q chromatography as described in Materials and Methods. [b] The percentage of each FTase peak was determined on the basis of the activities that eluted from the Mono-Q column.

chromatography to separate peaks I and II. Table 1 shows that another human tumor cell line, Calu I (a lung carcinoma), as well as NIH 3T3 cells contain an equal amount of FTases I and II. Furthermore, freshly isolated human tonsils also have a significant amount of FTase II (30%). On the other hand, lymphocytes isolated from human blood contain only a small proportion of FTase II (Table 1). We are presently characterizing other normal and malignant human tissues to further establish the prevalence of peak II.

Both Daudi FTase peaks retained their chromatographic properties after dialysis against EDTA. Repeated Mono-Q chromatography of each peak resulted in elution of each enzyme activity as a single peak at the expected ionic strengths, indicating that no deterioration, degradation, or transformation of one peak into the other had taken place (data not shown). These results suggest that the two protein FTase activities are carried out by distinct proteins having different overall charges. Mono-Q fractions corresponding

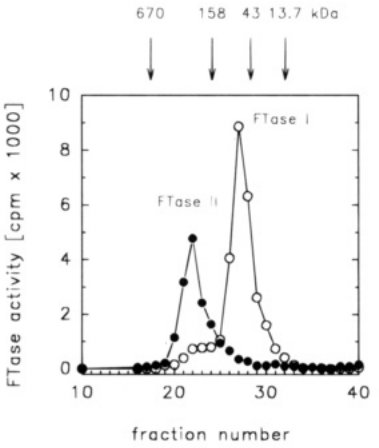

FIGURE 2: Gel filtration of Daudi FTases I and II. A 0.8 mg portion of Mono-Q-purified Daudi FTase I (○) or FTase II (●) was applied to a Superose 12 HR 10/30 gel filtration column. Elutions were carried out at 0.2 mL/min in 50 mM Tris (pH 7.5), 20 μM $ZnCl_2$, 1 mM DTT, and 200 mM NaCl. Fractions of 0.5 mL were collected and assayed for FTase activity as described in Materials and Methods. Arrows indicate the positions of known molecular mass standards: 670 kDa, 9.1 mL, thyroglobulin; 158 kDa, 12.4 mL, aldolase; 43 kDa, 13.7 mL, ovalbumin; 13.7 kDa, 16.1 mL, ribonuclease. Data are representative of three independent experiments.

to FTases I and II were pooled separately, concentrated by ultrafiltration, and used for further biochemical characterization.

The molecular masses of FTase I and II peaks were assessed by size-exclusion chromatography on a Superose 12 gel filtration column. Figure 2 is a combined profile from separate elutions of FTases I and II. We found that FTase I eluted from this column at a position corresponding to an apparent molecular mass of ca. 90 kDa, which compares well to literature data for rat brain FTase (Reiss et al., 1990). In a separate experiment under the same conditions, Daudi FTase I coeluted with FTase from rat brain (data not shown). In contrast, we found Daudi FTase II to be much larger; its elution position suggested an apparent molecular mass of ca. 250 kDa. Mono-Q-purified peak II was also dialyzed against EDTA for 2 days, run on a Superose 12 column, and shown not to dissociate into peak I (data not shown). These results further suggest that the two FTase activities found in Daudi cells are indeed derived from two distinct proteins.

The vast difference in molecular weight between FTases I and II prompted us to determine whether the two enzymes contained the α- and β-subunits of the previously reported FTase. Immunological characterization of FTases I and II was carried out at various stages of purification as described under Materials and Methods. Cytosolic fractions and fractions from Mono-Q- and Superose 12-purified FTases I and II were electrophoresed on SDS−PAGE, transferred to nitrocellulose, and immunoblotted with rabbit polyclonal antibodies directed against 19- and 17-mer peptides of the α- and β-subunits of rat brain FTase, respectively. Figure 3A shows that at all stages of purification, both FTases I and II show a single band that comigrated with the β-subunit from homogeneously purified rat brain FTase. This subunit has a molecular weight of ca. 50 kDa and is immunologically indistinguishable from rat brain FTase β-subunit. Figure 3B shows that FTase I contains an α-subunit that is also immunologically indistinguishable from rat brain FTase

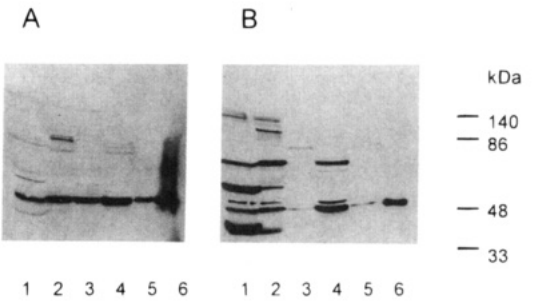

FIGURE 3: Western blot analysis of Daudi FTases I and II at various stages of purification. FTases from Daudi cells were purified by ion exchange chromatography and gel filtration as described in Materials and Methods. Samples containing 3.0 pmol of FPP/h of FTase activity were electrophoresed on 10% SDS−PAGE, transferred to nitrocellulose, and probed with antibodies directed against the α- and β-subunits of rat brain FTase (Santa Cruz Biotechnology): (A) detection of β-subunits and (B) detection of α-subunits; 1, Daudi 60S fractions; 2, Daudi FTase I, Mono-Q; 3, Daudi FTase II, Mono-Q; 4, Daudi FTase I, Superose; 5, Daudi FTase II, Superose; 6, homogeneously pure rat brain FTase. Data are representative of three independent experiments.

α-subunit. In contrast, FTase II reacted very poorly with an antibody against a peptide from the α-subunit of rat brain FTase. This suggested that Daudi FTase II may contain an α-subunit that is different from that in previously reported FTases. Since FTase II is much larger than FTase I, the β-subunit may also form a complex with one or more high molecular weight subunits of yet unknown nature. However, there were no bands detectable at the top of the gel that would indicate the presence of higher molecular weight proteins.

To date, only one form of FTase has been reported, and it was shown to be dependent on zinc and magnesium for enzymatic activity. Earlier substrate-binding assays had suggested that the enzyme contained zinc in its active site (Reiss et al., 1991a; Chen et al., 1993) and that both zinc and magnesium were required for the transfer of the farnesyl residue from a farnesylpyrophosphate−enzyme complex to a suitable acceptor protein (Reiss et al., 1992). We therefore determined whether Daudi FTases I and II were also dependent on these metal ions and found differences in their divalent cation requirements. The following experiment was performed using metal-free reagents and solutions that were carefully depleted from adventitious metal ions by dithizone treatment (Vallee & Galdes, 1984; Holmquist, 1988) as described in Materials and Methods. Extensive dialysis of Daudi FTases I and II from the Mono-Q step against metal-free buffers containing EDTA completely abolished FTase activity in both preparations (Figure 4). This effect was reversible by adding 3 mM $MgCl_2$ and 20 μM $ZnCl_2$ to the assay mixtures (Figure 4). These results are consistent with previous reports using EDTA dialysis to remove Zn^{2+} and Mg^{2+} from rat brain FTase (Reiss et al., 1992; Chen et al., 1993). In the absence of $MgCl_2$, both FTases I and II were inactive even at Zn^{2+} concentrations as high as 20 μM (Figure 4). However, the zinc requirements of FTases I and II in the presence of $MgCl_2$ were different. In the absence of Zn^{2+}, the addition of 3 mM $MgCl_2$ alone restored only 10% activity for FTase I whereas FTase II recovered over 30% of its maximum activity. Addition of up to 100 nM $ZnCl_2$ did not increase enzymatic activity of either peak (Table 2). However, the activity of both FTases increased at concentrations above 100 nM and saturated at 10 μM (Figure 4C,D).

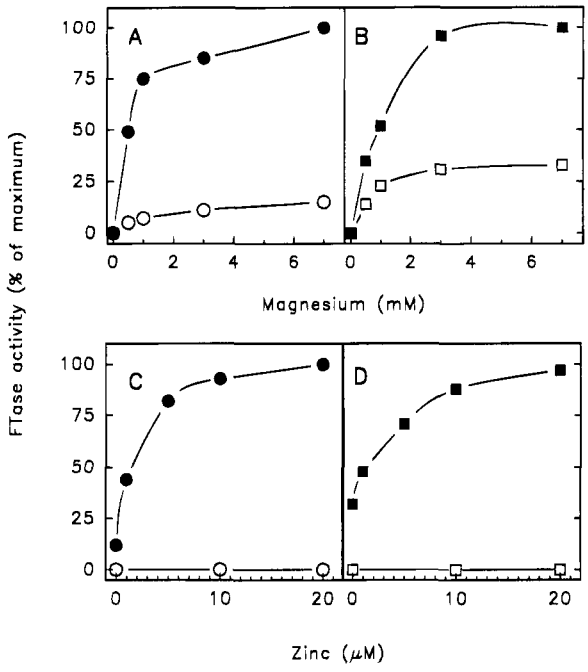

FIGURE 4: Divalent cation requirements of Daudi FTases I and II. Mono-Q-purified Daudi FTases were dialyzed for 3 days at 4 °C against dithizone-treated buffer containing EDTA and analyzed for FTase activity as described in Materials and Methods in the presence of various concentrations of Zn^{2+} and Mg^{2+}. Upper panel: Magnesium dependence of Daudi FTase I (A) and FTase II (B) activities in the presence (closed symbols) or absence (open symbols) of 20 μM Zn^{2+}. Lower panel: Zinc dependence of Daudi FTase I (C) and FTase II (D) activities in the presence (closed symbols) or absence (open symbols) of 3 mM Mg^{2+}. Data are expressed as a percentage of maximum, which was 0.75 pmol of FPP/h for FTase I and 0.7 pmol of FPP/h for FTase II (15 900 and 13 900 cpm/filter, respectively) at 20 μM $ZnCl_2$ and 7 mM $MgCl_2$, and are representative of three independent experiments.

Table 2: Activities of Metal-Free Daudi FTases I and II at Submicromolar Levels of $ZnCl_2$[a]

	FTase activity (% of maximum)		
$ZnCl_2$ (μM)	FTase I	FTase II	p
0	12.3 ± 1.9	32.3 ± 2.6	0.0007
0.01	12.0 ± 3.3	30.7 ± 2.5	0.0018
0.1	18.3 ± 4.6	36.0 ± 2.2	0.0094

[a] Data are the averages of duplicate determinations from three independent experiments and expressed in percent of maximum activity ±SEM. p-Values are derived from a student t-test. Assays were performed in the presence of 3 mM $MgCl_2$.

In order to confirm that p21 Ras binding did indeed occur in the absence of zinc, we conducted a substrate saturation study with H-Ras-CVLS in the presence and absence of $ZnCl_2$. Figure 5 shows that, in the presence of 50 μM $ZnCl_2$, there is little difference in the saturation characteristics of the two Daudi FTases, with H-Ras-CVLS having an apparent K_m of 8 μM (Figure 5, closed symbols). However, in zinc-depleted FTase preparations, little substrate binding was observed with Daudi FTase I. In contrast, FTase II bound p21 Ras with the same apparent K_m whether zinc was present or not (Figure 5B).

To confirm the specificity of zinc-independent protein substrate binding, we performed an inhibition study with two synthetic Ras CAAX peptidomimetics that we have recently developed and found to be potent inhibitors of FTase (Vogt et al., 1995). The CVIM peptidomimetics *N*-[4-[*N*-(3-

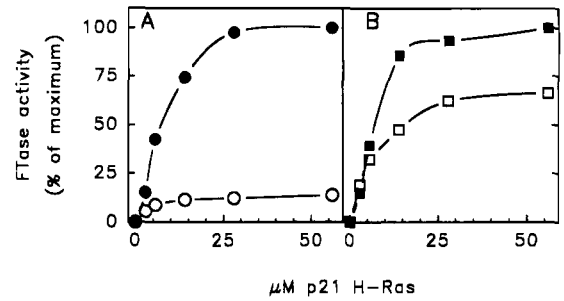

FIGURE 5: p21 Ras substrate saturation curves for Daudi FTases I and II in the presence and absence of zinc. Partially purified Daudi FTases were dialyzed for 3 days at 4 °C against EDTA. Aliquots were analyzed for FTase activity in the presence of the indicated concentrations of recombinant H-Ras-CVLS and 3 mM $MgCl_2$: (A) activity of Daudi FTase I in the absence (○) or presence (●) of 50 μM $ZnCl_2$ and (B) activity of Daudi FTase II in the absence (□) or presence (■) of 50 μM $ZnCl_2$. Data are representative of three independent experiments.

Table 3: Inhibition of Daudi FTases I and II by CAAX Peptidomimetics[a]

	$ZnCl_2$			
	50 μM		none	
	inhibitor IC_{50} (nM)			
	FTI-249	FTI-265	FTI-249	FTI-265
Daudi FTase I	190	70	ND[b]	ND[b]
Daudi FTase II	240	80	210	80

[a] Mono-Q-purified FTases from Daudi cells were mixed with appropriate amounts of the peptidomimetics and assayed for FTase activity in the presence and absence of $ZnCl_2$ as described in Materials and Methods. Data shown are the averages of at least two independent experiments. [b] Not determined. An inhibition study was not performed because Daudi FTase I is inactive in the absence of Zn^{2+}.

mercapto-2-aminopropyl)amino]benzoyl]methionine (FTI-249), in which the dipeptide VI was replaced by 4-aminobenzoic acid, and 4-[*N*-(3-mercapto-2-aminopropyl)amino]-3′-carboxybiphenyl (FTI-265), a nonpeptide mimetic in which the tripeptide VIM was replaced by biphenyl (Vogt et al., 1995), were used. It had been suggested that a zinc ion in the peptide-binding site plays an important role in bringing close together the cysteine thiol and the free carboxylate of the C-terminal methionine, resulting in a β-turn conformation of the peptide (James et al., 1993). Table 3 shows that both peptidomimetics inhibited FTases I and II equally well. Furthermore, there was also no difference in the ability of the peptidomimetics to inhibit FTase II in the absence of Zn^{2+}, suggesting that Zn^{2+} is not required for CAAX peptidomimetics to bind and inhibit FTase II. This is consistent with our recent reports that showed that peptidomimetics FTI-249 and FTI-265 can not take up a β-turn conformation that would result in Zn^{2+} complex formation. Structural details of these peptidomimetics and the lack of β-turn conformation are further discussed in Vogt et al. (1995) and Qian et al. (1994a,b).

The data presented here indicate that Burkitt lymphoma Daudi cells contain two distinct FTases that are different in charge, size, immunochemical behavior, and divalent cation requirements. Whereas FTase I is similar to previously reported FTases found in other mammalian tissues, FTase II shows distinct biochemical differences. We have documented its presence in cultured cells as well as in normal human tissues. We are currently investigating the physi-

ological functions of the two FTases and their relative contributions in farnesylating various cellular substrates.

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