Inhibitors of farnesyl-protein transferase (FPTase) show promise as anticancer agents. Based on the sequence of the protein substrates of FPTase (the CAAAX sequence), potent and selective peptidomimetic inhibitors have been developed; these compounds share with the peptide substrate a free thiol and a C-terminal carboxylate. We have used a synthetic four-amino acid peptide library to screen for new leads devoid of these features; the peptides were C-terminally amidated, and no free thiol was included in the combinatorial building blocks. To compensate for this negative bias, an expanded set of 68 amino acids was used, including both L and D as well as many non-coded residues. Sixteen individual tetrapeptides derived from the consensus were synthesized and tested; all were active, showing IC\textsubscript{50} values ranging from low micromolar to low nanomolar. The most active peptide, d-tryptophan-d-methionine-d-4-chlorophenylalanine-L-\gamma-carboxyglutamic acid (K\textsubscript{D} = 2 mM), is also very selective showing little inhibitory activity against the related enzyme geranylgeranyl-protein transferase type I (IC\textsubscript{50} > 50 \mu M). In contrast to CAAAX-based peptidomimetics, d-tryptophan-d-methionine-d-4-chlorophenylalanine-L-\gamma-carboxyglutamic acid appeared to mimic the iso- prenoid substrate farnesyl diphosphate as determined by kinetic and physical measurements. d-Tryptophan-d-methionine-d-4-chlorophenylalanine-L-\gamma-carboxyglutamic acid was a competitive inhibitor of FPTase with respect to farnesyl diphosphate substrate and uncompetitive with respect to CAAAX substrate. Furthermore, we demonstrated that FPTase undergoes ligand dependent conformational changes in its circular dichroism spectrum and that d-tryptophan-d-methionine-d-4-chlorophenylalanine-L-\gamma-carboxyglutamic acid induced a conformational change identical to that observed with farnesyl diphosphate ligand.

The ability of the ras oncogene protein Ras to transform cells is dependent upon farnesylation, a key post-translational modification that is catalyzed by farnesyl-protein transferase (FPTase).\(^1\) The development of FPTase inhibitors is the focus of intense research, since these compounds have potential as anticancer agents (1–3). FPTase catalyzes the transfer of the farnesyl moiety (C\textsubscript{15}) of farnesyl diphosphate (FPP) to a cysteine thiol in protein substrates such as Ras having specific C-terminal CAAX sequences (where C is cysteine, A is aliphatic amino acid, and X is Ser or Met). Another enzyme, geranylgeranyl-protein transferase type I (GGPTase-I), catalyzes transfer of geranylgeranyl (C\textsubscript{20}) diphosphate to a cysteine residue of proteins in which the last residue of CAAX is typically a Leu residue (4, 5).

Based on the CAAX tetrapeptide, several peptidomimetic inhibitors have been developed that display nanomolar inhibitory potency toward FPTase while retaining selectivity against GGPTase-I (6–9). In addition, mimetics of the farnesyl diphosphate substrate have also been described (10). Some of these FPTase inhibitors have been used to demonstrate antitumorigenic activity against ras-transformed cells \textit{in vivo} and in animal models (7, 8).

The usefulness of combinatorial peptide libraries for drug discovery has been repeatedly shown (for a review, see Ref. 11). In all cases where the lack of information about the receptor structure prevents structure-based ("rational") design, new and often unexpected binding motifs may be found through selection. For example, the use of a synthetic peptide combinatorial library (SPCL) of acetylated hexapeptides enabled the selection of new ligands for the opioid receptor (termed "acetalins"), which were lacking the N-terminal amine, a feature known to be critical for binding (12). The peptidomimetic inhibitors of FPTase based on CAAX typically contain a free thiol and the C-terminal carboxylate or an ester as prodrug form (6–9). We show here that tetrapeptide inhibitors with comparable \textit{in vitro} activity and selectivity were obtained by the use of an SPCL (13) that is C-terminally amidated and is devoid of Cys. The selected peptides inhibit FPTase by interacting with the farnesyl diphosphate site and not the CAAX site of the enzyme.

**EXPERIMENTAL PROCEDURES**

**Peptide Synthesis**—All the protected amino acids are commercially available from Nova Biochem (Laufelfingen), Bachem (Bubendorf), or NeoSystem (Strasbourg); we used FMoc (N-(9-fluorenylmethoxycarbonyl)tert-butyl) protected derivatives. The SPCL and the individual peptides were synthesized as described previously (14, 15), using a Zinsser SMPS 350 multiple peptide synthesizer, PyBOP\(^1\)-hydroxybenzotriazole/diisopropyl ethylamine (1:2) activation, 5-fold excess, and a coupling time of 20 min to 2 h as judged by the standard ninhydrin and 2,4,6-trinitrobenzenesulfonic acid color tests. The undefined or "mixed" (X) positions were incorporated by coupling a mixture of activated amino acids, with the relative ratios suitably adjusted to compensate for the difference in reactivity (Table I). Near equivalence of the incorporation was assessed by quantitative amino acid analysis, using two standard amino acid mixtures (natural and uncoded) for calibration. Trp, labile to the acid hydrolysis conditions used (tube sealed under vacuum, azetropic HCI, 110 °C, 18 and 24 h), was not

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\(^1\) The abbreviations used are: FPTase, farnesyl-protein transferase; FPP, farnesyl diphosphate; GGPTase, geranylgeranyl-protein transferase; SPCL, synthetic peptide combinatorial library; Fmoc, p-chlorophenylalanine; Gla, \(\gamma\)-carboxyglutamic; Hph, homo-L-phenylalanine; Cha, \(\beta\)-cyclohexylalanine.
quantitated, Asn-Asp and Gln-Glu eluted as a single peak, and for a few of the 41 chromatographic peaks we could not achieve base-line separation; within these limits, we estimated the maximum deviation from equimolarity to be ±30%.

**FPTase and GGPTase-I Inhibition Assay**—The assays were performed using human FPTase or GGPTase expressed in *Escherichia coli* and purified as described (16). For FPTase, a typical reaction in a final volume of 50 μl contained 100 mM HEPES (pH 7.0), 0.25 mM MgSO₄, 0.2% at 222 and 215 nm, while changes seen upon ligand binding were significantly larger. CD the standard deviation from the mean was bandwidth of 1 nm, step resolution of 0.5 nm, and a scan speed of 10 mg/ml using a 0.01-cm path length thermal jacketed cylindrical quartz cuvette (Hellma). Spectra were collected from 260 to 184 nm, using a mg/ml containing [3H]geranylgeranyl diphosphate (100 nM; 19.3 Ci/mmol, DuPont NEN), Ras-CAIL (1 μM), 50 mM HEPES, pH 7.5, 5 mM MgCl₂, 5 mM ZnCl₂, and 1 mM FPTase. For GGPTase-I, a typical reaction in a final volume of 50 μl contained [3H]geranylgeranyl diphosphate (100 nM; 19.3 Ci/mmol, DuPont NEN), Ras-CAIL (1 μM), 50 mM HEPES, pH 7.5, 5 mM MgCl₂, 5 mM dithiothreitol, 1% polyethylene glycol 15,000, 100 μM ZnCl₂, and 1 mM FPTase. Reactions were initiated at 30 °C and stopped at 15 min by the addition of 10% HCl in ethanol (2 ml). The quenched reactionswere determined using the extinction coefficient (ligand:enzyme): (3S,4S)-4-amino-3-hydroxy-5-phenylpentanoic (AHPPA) 1
(3S,4S)-4-amino-3-hydroxy-5-cyclohexylpentanoic (ACHPA) 1
(3S,4S)-4-amino-3-hydroxy-6-methylheptanoic (Statine) 1
1,2,3,4-Tetrahydroisoquinoline-3-L-carboxylic (TIC 1.4)
1,2,3,4-Tetrahydroisoquinoline-3-D-carboxylic (tic) 1.4
1,2,3,4-Tetrahydroisoquinoline-3-N-carboxylic (nic) 1.4
1,2,3,4-Tetrahydroisoquinoline-3-O-carboxylic (nico) 1.4
1,2,3,4-Tetrahydroisoquinoline-3-S-carboxylic (sico) 1.4

for coupling) 1
2,3-Diamino-1-propionic (αDP, either α or β-NH₂ free for coupling) 1
1,2,3,4-Tetrahydroisoquinoline-3-L-carboxylic (TIC 1.4)
1,2,3,4-Tetrahydroisoquinoline-3-D-carboxylic (tic) 1.4
1,2,3,4-Tetrahydroisoquinoline-3-N-carboxylic (nic) 1.4
1,2,3,4-Tetrahydroisoquinoline-3-O-carboxylic (nico) 1.4
1,2,3,4-Tetrahydroisoquinoline-3-S-carboxylic (sico) 1.4

The indicated abbreviations refer to Figs. 1 and 2.

**RESULTS AND DISCUSSION**

**Identification of New FPTase Inhibitors**—Peptidomimetic inhibitors of FPTase designed from a CAAX tetrapeptide template typically contain a free thiol and a C-terminal carboxylate, which requires an ester prodrug strategy to render the inhibitors cell-active. Both the thiol and the prodrug may be metabolic liabilities to the activity of FPTase inhibitors in animals. To find new FPTase inhibitors devoid of these two features, we have screened an SPCL composed of 2 × 10⁷ C-terminally amidated tetrapeptides, which did not include any free thiol among the combinatorial building blocks. To compensate for the negative bias deriving from exclusion of two key FPTase binding motifs we used an expanded combinatorial set, including many non-coded amino acids, and most of the residues were present both with the L and D chirality. Overall, we used a total of 68 amino acids (Table I). The library was prepared in the so-called positional scanning format (18) because of the great practical advantage of using the same library in multiple screening assays (14, 18–20). For the tetrapeptide positional scanning SPCL we synthesized four different sublibraries, H-O₁ (1 μM), 50 mM HEPES, pH 7.5, 5 mM MgCl₂, 5 mM dithiothreitol, 1% polyethylene glycol 15,000, 100 μM ZnCl₂, and 10 mM GGPTase-I. Reactions were initiated at 30 °C and stopped at 15 min by the addition of 10% HCl in ethanol (2 ml). The quenched reactions were vacuum-filtered through Whatman GF/C filters. Filters were washed four times with 3-ml aliquots of 100% ethanol, mixed with scintillation fluid (10 ml), and counted in a Beckman LS8001 scintillation counter. IC₅₀ values were determined using a 5-point titration where the concentration of inhibitor was varied from 0.1 to 1000 nM. The individual peptides or peptide mixtures from the combinatorial library were prepared as 40 mM stock solutions in 100% dimethyl sulfoxide. The solutions were diluted in the assay buffer to the appropriate final concentration, yielding a final dimethyl sulfoxide concentration of 5%. Steady-state kinetic analyses to describe the patterns of inhibition for the identified compounds were performed as described previously (17).

The extinction coefficient was based on the tyrosine and tryptophan content because the disulfide composition of the protein has not been determined.

**Spectroscopic Analyses**—CD spectra (far UV, 260–184 nm) were recorded at 30 °C with a Jasco J720 spectropolarimeter that was calibrated with a 0.1% aqueous solution of 10-camphorsulfonic acid. The spectrum for FPTase was recorded at a protein concentration of 0.53 mg/ml using a 0.01-cm path length quartz cuvette (Hellma). Spectra were collected from 280 to 184 nm, using a bandwidth of 1 nm, step resolution of 0.5 nm, and a scan speed of 10 nm/min. Each spectrum represents an average of three scans from which the appropriate base line was subtracted. In three measurements the standard deviation from the mean was ±0.2% at 222 and 215 nm, while changes seen upon ligand binding were significantly larger. CD studies were conducted in 5 mM Na-HEPES, pH 7.0, 0.25 mM MgSO₄, 0.01 mM ZnSO₄, 0.5 mM dithiothreitol. Ligands were prepared in the same buffer and added to the enzyme at the following molar ratio (ligand:enzyme): (α-hydroxyfarnesylphosphonate L-704,272 (2:1), CVIM (3:1), and D-Trp-D-Met-D-Fcl-L-Gla-NH₂ (2:1). Spectra are presented as mean residue ellipticity, based on protein concentrations determined using the extinction coefficient ε<sub>FPTase</sub> 157,440 M⁻¹ cm⁻¹.
Peptide mixtures (O1 position)

**Fig. 1. Screening of the sublibrary H-O1 XXX-NH2 for inhibition of FPTase activity.** White bars, final concentration of the peptide mixtures is 2 mM (about 6 nM for each single peptide); black bars, final concentration is 0.4 mM. When both the L and D forms of an amino acid are present, the L-isomer is shown in upper case three-letter code, and the D-isomer in lower case (a list of abbreviations is given in Table I). Only the most active pools in the 2 mM screen were retested at 0.4 mM. The most reactive residues at the lower concentration are indicated by the arrows.

The peptide mixtures of the H-O2 XXX-NH2 sublibrary were screened for inhibition of FPTase activity *in vitro* at a final concentration of 2 mM (each peptide about 6 nM), and the results are shown in Fig. 1 (white bars). Since several pools gave >50% inhibition, the most active mixtures were re-screened at 0.4 mM (black bars). Under these conditions, D-Trp was the most active residue, followed by L-Hph. The other sublibraries (positions O2–O4) were then screened at 0.4 mM, and the results are shown in Fig. 2.

The consensus sequences derived from the screening were: position 1, D-Trp, L-Hph; position 2, D-Met, D-Phe, D-Trp, D-Tyr, D-Cha, L-Trp; position 3, D-Phe, D-Trp, D-Fcl, L-Fcl, L-Gla; position 4, L-Glu; D-Amino acids were preferred in the second and, to a lesser extent, in the third position, but the equivalent L-residues were quite active. The dicarboxylic acid Gla was by far the preferred residue in the fourth position; however, neither L/D-Asp nor Glu was active in this position. All the non-coded residues in our SPCL, as these form 4/4 residues in the FPTase assay under different substrate concentration conditions. A significant decrease in IC50 value was observed when the FPP concentration was lowered from 100 to 10 nM. Under these conditions the following IC50 values were observed: D-Trp-D-Met-D-Fcl-L-Gla-NH2 (1.3 nM); D-Trp-D-Met-D-Phe-L-Gla-NH2 (2.1 nM); D-Trp-D-Trp-D-Fcl-L-Gla-NH2 (12.9 nM). Upon increasing the FPP concentration in the assay to 2 μM, the IC50 value for D-Trp-D-Met-D-Fcl-L-Gla-NH2 was 1200 nM. In contrast, increasing or decreasing the Ras substrate concentration did not affect the IC50 values of these tested compounds. These results suggested that the tested compounds might be behaving as FPP mimetics rather than CaaX mimetics, and the most active peptide, D-Trp-D-Met-D-Fcl-L-Gla-NH2, was further investigated in this regard.

Steady-state kinetic analysis of the mechanism of inhibition of FPTase by D-Trp-D-Met-D-Fcl-L-Gla-NH2 showed that the compound was competitive with respect to Ras substrate (Fig. 3A) with a KI = 32 ± 8 nM (n = 4). In contrast, D-Trp-D-Met-D-Fcl-L-Gla-NH2 was found to be competitive with respect to FPP (Fig. 3B) with a KI = 2.0 ± 0.8 nM (n = 4). The steady-state inhibition patterns for D-Trp-D-Met-D-Fcl-L-Gla-NH2 are consistent with an ordered sequential mechanism of FPTase as previously reported for the mammalian and yeast enzymes (23–25). Since we were surprised that a peptide-based inhibitor was competitive with FPP, we further assessed whether D-Trp-D-Met-D-Fcl-L-Gla-NH2 was binding to the isoprenoid site of FPTase by using CD spectroscopy to correlate the mechanism of ligand binding with the FPTase conformational state.

**CD Spectroscopic Analysis of Free and Ligand-bound FPTase**—The far UV CD spectra of recombinant human FPTase displayed intense positive bands at 192 nm. The CD spectrum of FPTase exhibited a broad minimum centered at 192 nm. To evaluate whether FPTase undergoes ligand-dependent conformational changes, we first tested the effects of FPTase substrates.

The specific substrate tetrapeptide CVIM was utilized as protein ligand, and (α-hydroxyfarnesyl)phosphonate (L-704,272) was used as an FPP analog to prevent product formation during the experiments. L-704,272 is a competitive inhibitor of farnesyl diphosphate and a noncompetitive inhibitor of Ras-CVLS (17). Neither CVIM nor L-704,272 contributed to the far UV CD spectrum at the concentrations utilized in the study. CVIM also did not display absorbance between 184 and 190 nm. Furthermore, the CD spectrum of the ternary complex of 150-fold IC50 for position 3 (compare peptides 9 and 13). Overexpression of the fusion protein inhibitor was competitive with respect to FPP, we further assessed whether D-Trp-D-Met-D-Fcl-L-Gla-NH2 was binding to the isoprenoid site of FPTase by using CD spectroscopy to correlate the mechanism of ligand binding with the FPTase conformational state.

| Position 1 | Position 2 | Position 3 | Position 4 |
|------------|------------|------------|------------|
| D-Trp      | D-Met      | D-Phe      | D-Trp      |
| D-Cha      | L-Trp      | D-Phe      | D-Trp      |
| D-Trp      | D-Fcl      | L-Fcl      | L-Gla      |
| D-Phe      | D-Trp      | D-Fcl      | L-Glu      |
| L-Hph      | L-Trp      | L-Phe      | L-Trp      |
| L-Glu      | L-Trp      | L-Phe      | L-Trp      |
| L-Fcl      | L-Trp      | L-Phe      | L-Trp      |
| L-Gla      | L-Trp      | L-Phe      | L-Trp      |
| L-Glu      | L-Trp      | L-Phe      | L-Trp      |
| L-Fcl      | L-Trp      | L-Phe      | L-Trp      |
| L-Gla      | L-Trp      | L-Phe      | L-Trp      |
| L-Glu      | L-Trp      | L-Phe      | L-Trp      |
| L-Fcl      | L-Trp      | L-Phe      | L-Trp      |
| L-Gla      | L-Trp      | L-Phe      | L-Trp      |
| L-Glu      | L-Trp      | L-Phe      | L-Trp      |
| L-Fcl      | L-Trp      | L-Phe      | L-Trp      |
| L-Gla      | L-Trp      | L-Phe      | L-Trp      |
| L-Glu      | L-Trp      | L-Phe      | L-Trp      |
| L-Fcl      | L-Trp      | L-Phe      | L-Trp      |
| L-Gla      | L-Trp      | L-Phe      | L-Trp      |
| L-Glu      | L-Trp      | L-Phe      | L-Trp      |
| L-Fcl      | L-Trp      | L-Phe      | L-Trp      |
| L-Gla      | L-Trp      | L-Phe      | L-Trp      |
| L-Glu      | L-Trp      | L-Phe      | L-Trp      |
| L-Fcl      | L-Trp      | L-Phe      | L-Trp      |
| L-Gla      | L-Trp      | L-Phe      | L-Trp      |
| L-Glu      | L-Trp      | L-Phe      | L-Trp      |
| L-Fcl      | L-Trp      | L-Phe      | L-Trp      |
| L-Gla      | L-Trp      | L-Phe      | L-Trp      |
| L-Glu      | L-Trp      | L-Phe      | L-Trp      |
| L-Fcl      | L-Trp      | L-Phe      | L-Trp      |
| L-Gla      | L-Trp      | L-Phe      | L-Trp      |
| L-Glu      | L-Trp      | L-Phe      | L-Trp      |
| L-Fcl      | L-Trp      | L-Phe      | L-Trp      |
260 nm at the concentrations utilized. Absorption flattening was, therefore, not a factor, as the protein absorbance was below 0.9 absorbance unit in the region of interest.

CD spectroscopy indicated that FPTase underwent a conformational change to an intermediate structure (ECAX) upon binding the peptide CVIM as demonstrated by a decrease in the mean residue ellipticity in the 208–222-nm region (Fig. 4A). Subsequent addition of L-704,272 resulted in a further conformational change to the structure (ECAX-L-704,272). The conformational change to ECAX-L-704,272 was manifested as a further decrease in ellipticity in the 212–224-nm region and an increase in ellipticity at 208 nm. Addition of L-704,272 to FPTase resulted in a more pronounced decrease in ellipticity in the 212–225-nm region than seen with CVIM alone, and the intermediate structure

Fig. 2. Screening of the sublibraries H-XOXX-NH₂, H-XXOXX-NH₂, and H-XXOXOXX-NH₂ for inhibition of FPTase activity. The final concentration was 0.4 mM (about 1 nM for each single peptide). The defined position (O₂–O₄) is indicated under each graph. The other details are as described in the legend to Fig. 1.
(E<sub>L-704,272</sub>) was different from the E<sub>CALX</sub> intermediate (Fig. 4A). Subsequent addition of CVIM to E<sub>L-704,272</sub> generated a spectrum superimposable on the spectrum seen with addition of the substrates in the opposite order (not shown). The order of ligand addition did not affect the final structure of the FPTase enzyme (E<sub>CALX-L-704,272</sub> or E<sub>L-704,272-CALX</sub>) but did determine which discrete intermediate structure FPTase adopted. In addition to observed spectral differences in the 208–225 nm region, small ligand-dependent changes in both the shape and position of the maxima centered at 192 nm were reproducibly observed (not shown). Secondary structure analysis of these data will be described elsewhere. We conclude from the above results that one

**TABLE II**

| Peptide                                      | IC<sub>50</sub> (nM) | FPTase<sup>a</sup> | GGPTase<sup>b</sup> |
|----------------------------------------------|-----------------------|--------------------|---------------------|
| 1. D-Trp-D-Met-D-Fcl-L-Gla-NH<sub>2</sub>     | 42                    | 62                 | >50,000             |
| 2. D-Trp-D-Met-D-Phe-L-Gla-NH<sub>2</sub>     | 50                    | 50                 | >11,000             |
| 3. D-Trp-D-Trp-D-Fcl-L-Gla-NH<sub>2</sub>     | 90                    | 90                 | NT<sup>b</sup>      |
| 4. D-Trp-D-Phe-D-Fcl-L-Gla-NH<sub>2</sub>     | 100                   | 100                | NT                  |
| 5. D-Trp-D-Met-D-Trp-L-Gla-NH<sub>2</sub>     | 110                   | 110                | NT                  |
| 6. D-Trp-D-Phe-D-Trp-L-Gla-NH<sub>2</sub>     | 160                   | 160                | NT                  |
| 7. D-Trp-D-Trp-D-Trp-L-Gla-NH<sub>2</sub>     | 330                   | 330                | NT                  |
| 8. D-Trp-D-Trp-D-Phe-L-Gla-NH<sub>2</sub>     | 500                   | 500                | NT                  |
| 9. D-Trp-D-Trp-D-Trp-L-Gla-NH<sub>2</sub>     | 700                   | 700                | NT                  |
| 10. D-Trp-D-Trp-D-Trp-L-Gla-NH<sub>2</sub>    | 880                   | 880                | NT                  |

<sup>a</sup> Assays performed as described under “Experimental Procedures.”

<sup>b</sup> NT, not tested.

**Fig. 3.** Inhibition of FPTase with D-Trp-D-Met-D-Fcl-L-Gla-NH<sub>2</sub>. A, double-reciprocal plot with Ras-CVLS as the varied substrate at fixed concentrations of D-Trp-D-Met-D-Fcl-L-Gla-NH<sub>2</sub>. Concentrations of inhibitor were 0 (○), 2.5 (●), 4.0 (□), 10.0 (■), 40.0 (◇), and 150 (●) nM. B, double-reciprocal plot with FPP as the varied substrate at fixed concentrations of D-Trp-D-Met-D-Fcl-L-Gla-NH<sub>2</sub>. Concentrations of inhibitor were 0 (○), 0.4 (●), 1.3 (□), 4.0 (■), 13.0 (◇), 50.0 (○), and 150 (●) nM. These results are representative of four independent experiments.

**Fig. 4.** CD spectra (far UV, 230–204 nm) of human FPTase recorded in the presence of ligands. A, the spectra of FPTase (——) were recorded as described under “Experimental Procedures” with the addition of the tetrapeptide CVIM at a 3:1 molar ratio (peptide:FPTase, --), L-704,272 at a 2:1 molar ratio (phosphonate:FPTase, --), or both (——). Each spectrum represents an average of three scans with the baseline subtracted and protein concentration corrected. B, the spectra of FPTase (——) were recorded with the addition of the tetrapeptide CVIM at a 3:1 molar ratio (peptide:FPTase, --), D-Trp-D-Met-D-Fcl-L-Gla-NH<sub>2</sub> at a 2:1 molar ratio (peptide:FPTase, --), or both (——). Each spectrum represents an average of three scans with the baseline subtracted and the protein concentration corrected.

(E<sub>L-704,272</sub>) was different from the E<sub>CALX</sub> intermediate (Fig. 4A). Subsequent addition of CVIM to E<sub>L-704,272</sub> generated a spectrum superimposable on the spectrum seen with addition of the substrates in the opposite order (not shown). The order of ligand addition did not affect the final structure of the FPTase enzyme (E<sub>CALX-L-704,272</sub> or E<sub>L-704,272-CALX</sub>) but did determine which discrete intermediate structure FPTase adopted. In addition to observed spectral differences in the 208–225 nm region, small ligand-dependent changes in both the shape and position of the maxima centered at 192 nm were reproducibly observed (not shown). Secondary structure analysis of these data will be described elsewhere. We conclude from the above results that one
can distinguish ligands that bind to either or both the CAAX and FPP sites of FPTase.

Upon addition of d-Trp-d-Met-d-Fcl-l-Gla-NH₂ to FPTase, the enzyme underwent a conformational change that was identical to that observed with L-704,272 (Fig. 4B). We specifically determined that the spectra obtained with these two ligands were superimposable (not shown). The alcohol farnesol, which lacks the dihydroxymoiety, had no effect on the CD spectrum of FPTase. Further control experiments comparing the CD spectrum of (α-hydroxyfarnesyl)phosphonate-bound FPTase (E₅₇₀nm=272) to FPTase bound to its natural substrate, FPP, revealed that they were identical over the entire wavelength range (data not shown). Neither the non-cysteine-containing tetrapeptide SVLS (the non-thiol equivalent of the FPTase substrate CVLS) nor another peptide, KCIF, had an effect on the conformation of FPTase. These results indicate that the observed conformational changes observed with d-Trp-d-Met-d-Fcl-l-Gla-NH₂ are not a general effect of peptides and provide further evidence that the peptide binds to the FPP site of FPTase.

CONCLUSIONS

While several microbial FPTase inhibitors have been identified that act as FPP competitors (10), none of these is of peptide nature. The compounds selected from the SPCL such as d-Trp-d-Met-d-Fcl-l-Gla-NH₂ thus represent a new class of inhibitors, opening the way to the synthesis of FPP peptidomimetics. The dicarboxylate of Gla most likely serves as a surrogate for the dicarboxylate-containing natural product (26). However, our initial analyses of these compounds in cells suggest that they are not cell-active as assayed by inhibition of Ras processing. This result suggests that additional modifications to these peptides will be necessary to render them biologically active. As a first step in this direction, we have determined the structure of one of the selected peptides bound to FPTase by transfer nuclear Overhauser effect experiments. At this stage, the work presented here has already demonstrated that FPTase is amenable to screening of combinatorial libraries, which can be used to identify highly potent and novel compounds.

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