Yeast Protein Geranylgeranyltransferase Type-I: Steady-State Kinetics and Substrate Binding[†]

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ABSTRACT: Protein geranylgeranyltransferase type-I (PGGTase-I) catalyzes alkylation of the cysteine residue in proteins containing a consensus C-terminal CaaX sequence ending in Leu or Phe by the C_{20} hydrocarbon moiety in geranylgeranyl diphosphate (GGPP). A kinetic study of the alkylation reaction was conducted with a continuous assay based on the fluorescence enhancement that accompanies geranylgeranylation of dansyl-GCIIL. The kinetic constants $k_{cat} = 0.34 \pm 0.01\ s^{-1}$, $K_M^G = 0.86 \pm 0.05\ \mu M$ for GGPP, and $K_M^D = 1.6 \pm 0.1\ \mu M$ for dansyl-GCIIL were calculated from initial rates measured at varying concentrations of the substrates. Inhibitor studies were conducted with dead-end inhibitors for GGPP and the peptide substrate. Double reciprocal plots for the peptide mimic Cys-AMBA-Leu gave a competitive pattern when plotted against varying concentrations of dansyl-GCIIL and an uncompetitive pattern against GGPP. Similar plots for 1-phosphono-(*E*,*E*,*E*)-geranylgeraniol, a dead-end inhibitor for GGPP, gave a competitive double reciprocal plot for varied concentrations of GGPP and induced potent substrate inhibition by dansyl-GCIIL when dansyl-GCIIL was the varied substrate. The dissociation constant (K_D) for the PGGTase-I·GGPP complex was 120 ± 20 nM. These results are consistent with an ordered binding mechanism for PGGTase-I where GGPP adds before peptide.

The posttranslational modification of proteins in eukaryotic cells with hydrophobic C_{15} farnesyl or C_{20} geranylgeranyl isoprenoid units is a common phenomenon. The modification involves alkylation of a cysteine residue near the C-terminus of the proteins to form a stable thioether and confers lipophilic properties to the proteins essential for their biological activity. Prenylated proteins fulfill a variety of important roles, including those in signal transduction and regulation of vesicular traffic (for a recent review, see Zhang & Casey, 1996).

Three protein prenyltransferases, a protein farnesyltransferase (PFTase),[1] a protein geranylgeranyltransferase type-I (PGGTase-I), and a protein geranylgeranyltransferase type-II (PGGTase-II), catalyze the alkylation reactions. PFTase and PGGTase-I modify cysteine residues in carboxy-terminal CaaX motifs in which "a" is an aliphatic amino acid, and "X" is one of several possible residues. When "X" is Ala, Met, Ser, Cys, or Gln, the protein substrate is farnesylated, and when "X" is Leu or Phe, the protein is geranylgeranylated (Moores et al., 1991; Reiss et al., 1991; Yokoyama et al., 1991; Omer et al., 1993; Caplin et al., 1994). Protein substrates for PFTase include fungal mating factors, nuclear lamins, and Ras G-proteins (Maltese, 1990; Moores et al., 1991; Clark, 1992). Substrates for PGGTase-I include the γ subunit of neural G-proteins and several Ras-related G-proteins (Maltese, 1990; Mumby et al., 1990; Kawata et al., 1990; Casey et al., 1991; Yamane et al., 1991). Following prenylation, the carboxy-terminal aaX tripeptide is removed, and the new carboxy-terminal cysteine is often methylated. PGGTase-II, often called Rab geranylgeranyltransferase, catalyzes alkylation of both cysteine residues in Rab proteins containing carboxy-terminal CC, CXCX, or CXC motifs (Seabra et al., 1992; Farnsworth et al., 1994).

All three protein prenyltransferases are α/β dimers, and each shares a high degree of similarity at the amino acid level. PFTase and PGGTase-I contain a common α subunit in combination with distinctive β subunits, which confer different selectivities for the two enzymes toward their isoprenoid and protein substrates. For PGGTase-II, an escort protein to bring the protein substrate to the catalytic heterodimer and to escort the geranylgeranylated product to membrane is required in addition to the catalytic heterodimer (Andres et al., 1993; Fujimura et al., 1994; Jiang & Ferro-Novick, 1994).

There is considerable interest in understanding how protein prenyltransferases bind their substrates because of the prominent roles modified proteins play in eukaryotic cells, including regulation of cell division and control of vesicle fusion (Gibbs et al., 1994). In kinetic studies with bovine PFTase, it was suggested that the enzyme binds substrates by a random mechanism (Pompliano et al., 1992), but with a preference for adding FPP before peptide (Pompliano et al., 1993). However, recent studies with the human (Furfine et al., 1995) and yeast (Dolence et al., 1995) forms of PFTase support an ordered addition of substrates.

PGGTase-I has been purified from bovine brain (Moomaw & Casey, 1992; Yokoyama & Gelb, 1993), and the yeast (Mayer et al., 1992; Stirtan & Poulter, 1995), rat, and human (Zhang et al., 1994a,b) enzymes have been purified from recombinant organisms. Steady-state kinetic studies with

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[1] Abbreviations: PFTase, protein farnesyltransferase; PGGTase-I, protein geranylgeranyltransferase type-I; PGGTase-II, protein geranylgeranyltransferase type-II; FPP, farnesyl diphosphate; GGPP geranylgeranyl diphosphate; 1-P-GGOH, 1-phosphono-(*E*,*E*,*E*)-geranylgeraniol; dansyl-GC^{GG}IIL, dansyl-Gly-Cys(S-geranylgeranyl)-Ile-Ile-Leu; AMBA, 3-(aminomethyl)benzoic acid; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; DMSO, dimethyl sulfoxide; TLC, thin-layer chromatography; DTT, dithiothreitol.

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mammalian PGGTase-I and reversible dead-end inhibitors suggested that the substrate binding mechanism was random (Zhang et al., 1994b; Yokoyama et al., 1995) but, like PFTase, appeared to be "functionally" ordered with geranylgeranyl diphosphate (GGPP) adding before the protein substrate (Yokoyama et al., 1995). Recently, we described the purification of recombinant yeast PGGTase-I and a continuous fluorescence assay to monitor enzyme activity (Stirtan & Poulter, 1995). We now present steady-state kinetic and inhibition studies which demonstrate that yeast PGGTase-I binds substrates to give a catalytically competent ternary complex by an ordered mechanism with no evidence of a random component.

MATERIALS AND METHODS

Materials. [^{3}H]GGPP (19.3 Ci mmol^{-1}) was purchased from DuPont New England Nuclear Research Products (Boston, MA). Unlabeled GGPP was synthesized from geranylgeranyl bromide and tris(tetrabutylammonium) pyrophosphate (Davisson et al., 1986). *n*-Dodecyl β-D-maltoside was purchased from Calbiochem, and dansylglycine was from Sigma (St. Louis, MO). Dansyl-GCIIL was prepared by solid-phase synthesis methods on an ABI peptide synthesizer Model 431A. 1-Phosphono-(*E*,*E*,*E*)-geranylgeraniol (1-P-GGOH) was prepared as described previously (Yokoyama et al., 1995). Cys-AMBA-Leu (Nigam et al., 1993) was provided by Dr. Andrew Hamilton. Recombinant yeast PGGTase-I was expressed in *Escherichia coli* (JM101/pWGS-1−237B) and purified as described previously (Stirtan & Poulter, 1995). Fluorescence data were collected on a Spex FluoroMax spectrofluorimeter. Quartz cuvettes were purchased from NSG Precision Cells Inc.

Methods. Preparation of Solutions. Stock solutions of GGPP (10−15 mM) were prepared in 25 mM NH_4HCO_3, and concentrations of GGPP were determined by phosphate analysis (Reed and Rilling, 1976). Stock solutions of dansyl-GCIIL (~300 μM) were prepared by dissolving the dansylated peptide in peptide buffer (50 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 5 mM DTT, 0.020% *n*-Dodecyl β-D-maltoside) or with detergent-free peptide buffer for inhibition studies. The concentration of dansyl-GCIIL was determined from a standard curve of A_{340} versus the concentration of dansylglycine, in the same buffer. Stock solutions of dansyl-GC^{GG}IIL, the geranylgeranylated product of the PGGTase-I reaction, were prepared and used in calibration studies exactly as described previously (Stirtan & Poulter, 1995).

Prenyltransferase Assay. PGGTase-I was assayed using a continuous fluorescence assay based on the large fluorescence enhancement which accompanies geranylgeranylation of a dansylated pentapeptide (Pickett et al., 1995; Stirtan & Poulter, 1995). Assays were carried out at 30 °C in prewarmed cuvettes (3 mm^2) using a thermostated cuvette holder. Buffer concentrations in the final assay volume (220 μL) for all kinetic studies were 50 mM Tris-HCl, pH 7.5, 5.0 mM DTT, 1.0 mM $MgCl_2$, 10 μM $ZnCl_2$, 0.020% *n*-dodecyl β-D-maltoside. Typically, solutions of dansyl-GCIIL (10 μL) and GGPP (10 μL) were added to assay buffer (53 mM Tris-HCl, pH 7.5, 5.3 mM DTT, 1.1 mM $MgCl_2$, 11 μM $ZnCl_2$, 0.021% *n*-Dodecyl β-D-maltoside) (190 μL). The mixture was preincubated at 30 °C for 5 min before the reaction was initiated with enzyme (10 μL, ~180 ng) previously diluted with assay buffer to the appropriate concentration. The sample was vigorously mixed, a 200 μL-portion of the reaction was immediately transferred to a prewarmed cuvette, and the fluorescence intensity was measured for 5 min. Excitation and emission wavelengths were 340 and 486 nm, respectively, with a bandpass of 5.1 nm for both excitation and emission monochromators, unless otherwise noted. The rate of change in fluorescence intensity (cps s^{-1}) was converted to units of velocity (μM s^{-1}) given the following conversion factor ($m = 3.6 \times 10^6$ cps μM^{-1}) and fluorescence enhancement ($E = 10$) (Stirtan & Poulter, 1995).

Inhibition Studies. Stock solutions of 1-P-GGOH were prepared in DMSO. Stock solutions of Cys-AMBA-Leu were prepared in H_2O, and DTT was added to 5 mM. The concentration of Cys-AMBA-Leu was determined by thiol titration prior to the addition of DTT. For inhibition studies, GGPP solutions were prepared in 25 mM NH_4HCO_3/0.44% *n*-Dodecyl β-D-maltoside to reduce variation in duplicate measurements at low GGPP concentrations which can arise from GGPP adsorption to pipette tips and microcentrifuge tubes (Zhang et al., 1994b). Typically, solutions of GGPP (10 μL), dansyl-GCIIL (10 μL), and inhibitor (10 μL) were added to detergent-free assay buffer (180 μL), preincubated at 30 °C for 5 min, and initiated with PGGTase-I as described above. A bandpass of 6.4 or 7.6 nm was used in all inhibition studies. The conversion factor at each slit width was determined in separate calibration experiments as described previously (Stirtan & Poulter, 1995).

The ability of PGGTase-I to geranylgeranylate Cys-AMBA-Leu was examined using a TLC assay similar to that reported for PFTase (Goldstein et al., 1991). Cys-AMBA-Leu (230 μM) was incubated with [^{3}H]GGPP (110 mCi mmol^{-1}, 8.5 μM) and PGGTase-I (160 ng) in 50 mM Tris-HCl, pH 7.5, 5.0 mM DTT, 1.0 mM $MgCl_2$, 10 μM $ZnCl_2$, 0.020% *n*-Dodecyl β-D-maltoside (25 μL) for 30 min at 30 °C. The entire reaction mixture was spotted onto a 4 × 18 cm analytical aluminum-backed silica TLC plate (Whatman) and allowed to dry. The plate was eluted with *n*-propyl alcohol/ammonium hydroxide/water (6:3:1 v/v/v), and cut into 1 cm strips. Scintillation fluid (CtyoScint, (ICN)) (10 mL) was added, and the samples were counted for radioactivity.

Measurement of the Dissociation Constant for GGPP. The K_D for GGPP dissociating from the PGGTase-I·GGPP complex was determined at 30 °C using [^{3}H]GGPP (1 Ci mmol^{-1}) in buffer containing 50 mM Tris-HCl, pH 7.5, 5.0 mM DTT, 1.0 mM $MgCl_2$, 10 μM $ZnCl_2$, 0.020% *n*-Dodecyl β-D-maltoside. Solutions (100 μL) containing PGGTase-I (0.21 μM) and [^{3}H]GGPP (0.065−0.58 μM) were incubated at 30 °C for 30 min and then transferred to a Microcon-30 (Amicon). The sample was centrifuged (ca. 10 s), allowing a small volume (10−15 μL) to pass through the membrane. A portion (10 μL) was taken from the top compartment and counted to determine the total GGPP concentration. The free GGPP concentration was determined from the bottom compartment after correcting for membrane retention using a correction factor obtained in an identical experiment, except in the absence of enzyme. K_D was calculated by fitting the data to the following equation: [bound GGPP] = [E^t][free GGPP]/(K_D + [free GGPP]).

Equations for Analysis of Kinetic Data. For the mechanism shown in Scheme 1, the dependence of the steady-state velocity on substrate concentration for PGGTase-I is shown in eq 1, where *G* and *D* are the concentrations of

Scheme 1: An Ordered Bireactant Mechanism

$$E + G \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\text{-}G + D \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E\text{-}G\text{-}D \xrightarrow{k_3}$$

$$E\text{-}P_1\text{-}P_2 \xrightarrow{k_4} \text{products}$$

GGPP and dansyl-GCIIL, respectively. K_M^G and K_M^D are the K_M values for GGPP and dansyl-GCIIL at saturating second substrate concentration, respectively, K_a is the dissociation constant for GGPP, v is the velocity of product formation, and V is the maximal velocity of product formation.

$$v = \frac{VGD}{K_a K_M^D + K_M^D G + K_M^G D + GD} \quad (1)$$

A graphical analysis of double reciprocal plots was used to determine the mode of inhibition, and data were fit to the appropriate rate equation using a nonlinear regression analysis to obtain inhibition constants (Leatherbarrow, 1992). Equations 2 and 3 were used for competitive and uncompetitive inhibition. Data for substrate inhibition were fit to eq 4, where V, K, and K_I are apparent constants (Danenberg & Danenberg, 1978).

$$v = \frac{VA}{K(1 + I/K_{is}) + A} \quad (2)$$

$$v = \frac{VA}{K + A(1 + I/K_{ii})} \quad (3)$$

$$v = \frac{VA}{K + A + A^2/K_I} \quad (4)$$

RESULTS

Steady-State Kinetic Studies. Earlier kinetic studies with mammalian PGGTase-I suggested that the enzyme binds substrates by a mechanism which is formally random (Zhang et al., 1994b; Yokoyama et al., 1995) but has a kinetically preferred pathway in which GGPP binds before peptide (Yokoyama et al., 1995). Using dead-end reversible inhibitors, we found that yeast PGGTase-I bound GGPP before dansyl-GCIIL by an ordered mechanism with no evidence for a random component. We examined the steady-state mechanism for recombinant yeast PGGTase-I using a continuous fluorescence assay based on the large fluorescence enhancement that accompanies geranylgeranylation of the dansylated pentapeptide substrate dansyl-GCIIL (Stirtan & Poulter, 1995) to measure initial velocities. This assay is more precise than single point assays that measure incorporation of radioactivity into prenylated peptides. Steady-state kinetic constants were obtained by fitting hyperbolic plots of initial velocity versus GGPP (G) concentration, at several concentrations of dansyl-GCIIL (D) (Figure 1), to eq 1: $K_M^G = 0.94 \pm 0.08$ μM, $K_M^D = 1.8 \pm 0.2$ μM, $k_{cat} = 0.35 \pm 0.01$ s^{-1}, and $K_a = 14 \pm 80$ nM. Double reciprocal plots of the initial velocities versus the concentration of GGPP, at different concentrations of dansyl-GCIIL, were parallel over substrate concentrations between $0.5K_M$ and $6K_M$, consistent with $K_a \ll K_M^G$ (Segel, 1975).

Given the uncertainty in the calculated value for K_a, the dissociation constant for GGPP was determined in a separate

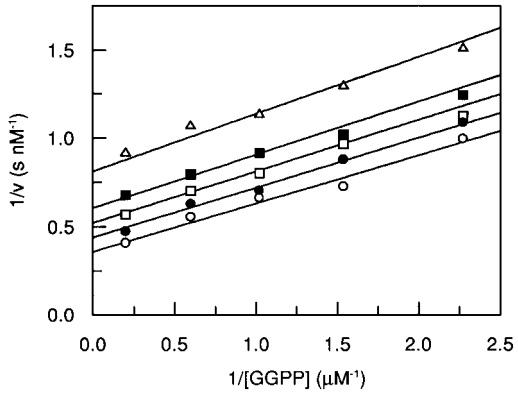
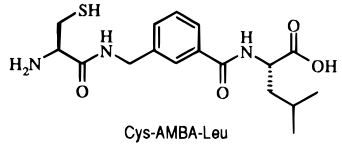


FIGURE 1: A double reciprocal plot of initial velocities versus GGPP concentrations at fixed concentrations of dansyl-GCIIL. The concentration of GGPP was varied between 0.5 and 5 μM at several dansyl-GCIIL concentrations: 9.5 (○), 3.8 (●), 2.3 (□), 1.7 (■), and 1.0 μM (Δ). Assays were carried out in duplicate under standard assay conditions and lines were calculated using the appropriate equation (see Materials and Methods).

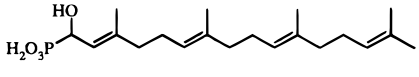
experiment using a microfiltration assay. The measured value for $K_D = 120 \pm 20$ nM is similar to that reported previously for the yeast PFTase·FPP complex (Dolence et al., 1995) and is significantly larger than that observed for mammalian PGGTase-I (Yokoyama et al., 1995). If K_a in eq 1 is fixed at the experimentally determined K_D for GGPP (120 nM), a very modest change in the calculated steady-state kinetic constants is observed: $K_M^G = 0.86 \pm 0.05$ μM, $K_M^D = 1.6 \pm 0.1$ μM, and $k_{cat} = 0.34 \pm 0.01$ s^{-1}.

Kinetic Studies with Dead-End Reversible Inhibitors. Compounds that are dead-end inhibitors for GGPP and dansyl-GCIIL were used to distinguish between random and ordered binding mechanisms for addition of substrates to PGGTase-I. Since uncompetitive inhibition is not observed with substrate analogs possessing significant activity (Spector and Cleland, 1981), a thin-layer chromatographic assay was used to confirm that Cys-AMBA-Leu (Nigam et al., 1993) was completely inert towards geranylgeranylation by PGGTase-I (data not shown). Double reciprocal plots of initial velocities for varied dansyl-GCIIL and GGPP concentrations at different fixed concentrations of Cys-AMBA-Leu are shown in Figure 2. When dansyl-GCIIL was the varied substrate, in the presence of a fixed concentration of GGPP, Cys-AMBA-Leu gave a competitive inhibition profile ($K_{is} = 78 \pm 8$ μM) (Figure 2a). When the concentration of GGPP was varied at fixed concentrations of dansyl-GCIIL, the data for inhibition by Cys-AMBA-Leu showed uncompetitive inhibition ($K_{ii} = 140 \pm 10$ μM) (Figure 2b), consistent with an ordered kinetic mechanism.

A hydroxyphosphonic acid analog of GGPP, 1-P-GGOH, was a competitive inhibitor of yeast PGGTase-I when GGPP



Cys-AMBA-Leu



1-P-GGOH

was varied (Figure 3a). The inhibition constant for 1-P-GGOH ($K_{is} = 0.20 \pm 0.05$ μM) is similar to the value

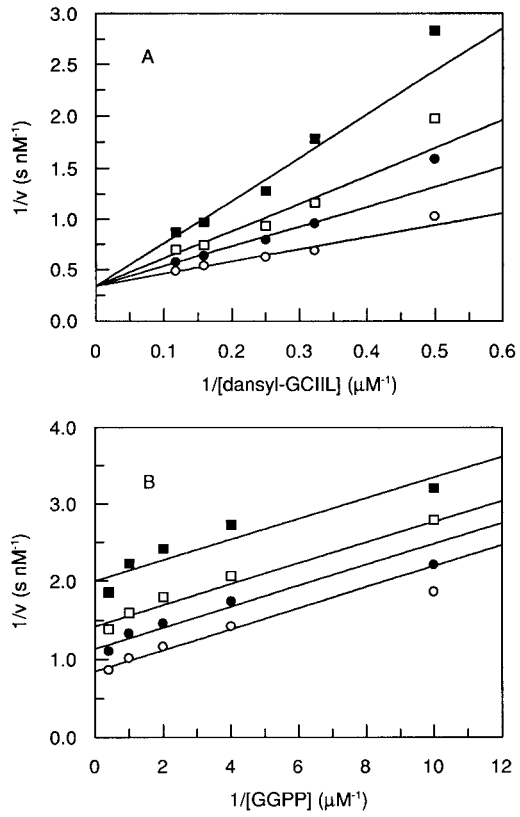


FIGURE 2: Inhibition of PGGTase-I by Cys-AMBA-Leu. (A) A double reciprocal plot of initial velocity versus dansyl-GCIIL concentration at 0 (○), 50 (●), 100 (□), 200 μM (■) Cys-AMBA-Leu and a fixed unsaturating concentration of GGPP (1.2 μM). (B) A double reciprocal plot of initial velocity versus GGPP concentration at 0 (○), 50 (●), 100 (□), or 200 μM (■) Cys-AMBA-Leu and a fixed unsaturating concentration of dansyl-GCIIL (2.9 μM). Each assay was performed in duplicate and the calculated lines were obtained using the appropriate equation (see Materials and Methods).

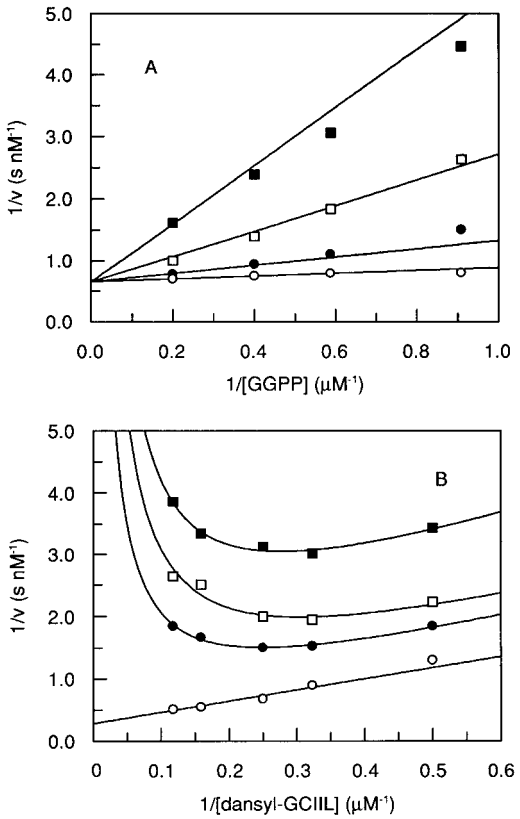
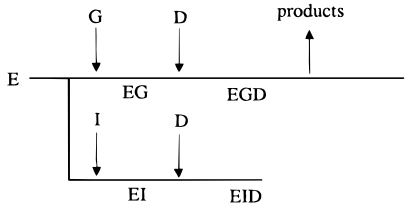


FIGURE 3: Inhibition of PGGTase-I by 1-P-GGOH. (A) A double reciprocal plot of initial velocity versus GGPP concentration at 0 (○), 0.40 (●), 1.6 (□), or 4.0 μM (■) 1-P-GGOH and a fixed unsaturating concentration of dansyl-GCIIL (4.0 μM). (B) A double reciprocal plot of initial velocity versus dansyl-GCIIL concentration at 0 (○), 7.7 (●), 15 (□), or 30 μM (■) 1-P-GGOH and a fixed unsaturating concentration of GGPP (1 μM). The apparent Michaelis and inhibition constants for dansyl-GCIIL (K_M^D and K_i^D) at the various 1-P-GGOH concentrations, obtained from eq 4 are as follows: 0 μM 1-P-GGOH, $K_M^D = 6.3$ μM; 7.7 μM 1-P-GGOH, $K_M^D = 10$ μM, $K_i^D = 1.6$ μM; 15 μM 1-P-GGOH, $K_M^D = 6.2$ μM, $K_i^D = 1.7$ μM; 30 μM 1-P-GGOH, $K_M^D = 3.8$ μM, $K_i^D = 3.4$ μM. Each assay was performed in duplicate and the calculated lines were obtained using the appropriate equation (see Materials and Methods).

obtained for the mammalian enzyme (Yokoyama et al., 1995). Furthermore, in the presence of unsaturating concentrations of GGPP, 1-P-GGOH induced substrate inhibition by dansyl-GCIIL (Figure 3b).

These results are consistent with an ordered mechanism, where the dead-end inhibitor that is competitive with the first substrate induces substrate inhibition by the second substrate (Fromm, 1967; Danenberg & Danenberg, 1978; Cleland, 1990). This scenario is illustrated for the ordered mechanism shown in Scheme 2, where dansyl-GCIIL (D) binds to the enzyme•1-P-GGOH complex to form an inactive ternary E•I•D complex. An increase in the concentration of dansyl-GCIIL (D) increases the concentration of the E•I•D complex. Since binding and, by the principle of microscopic reversibility, dissociation is ordered, the inhibitor is unable to dissociate prior to D. Thus, the amount of enzyme available to bind GGPP (G) is reduced. Conversely, in a random mechanism where the dissociation of each substrate is unaffected by the other substrate, induced substrate inhibition is not observed since the inhibitor would be free to dissociate from the inactive E•I•D complex to form the productive complex E•D. We were unable to extract individual inhibition constants from this data using the full rate expression describing the induced substrate inhibition (Danenberg & Danenberg, 1978; Cleland, 1990) shown in Scheme 2. Apparent Michaelis and inhibition constants for dansyl-GCIIL (K_M^D and K_i^D), for different fixed concentrations of

Scheme 2: An Ordered Bireactant Mechanism with Induced Substrate Inhibition



1-P-GGOH, were obtained from fitting the data in Figure 3b to eq 4 and are given in the figure legend. A plot of $1/v$ versus the dansyl-GCIIL concentration, in the region of the induced substrate inhibition (3−9 μM dansyl-GCIIL) at 1 μM GGPP and 30 μM 1-P-GGOH is linear (data not shown), consistent with the prediction from Scheme 2 that there is a high degree of order in this reaction and that the rate for the reaction approaches zero as the concentration of dansyl-GCIIL is raised. Conversely, a significant contribution from a random pathway would result in partial substrate inhibition and a hyperbolic plot of $1/v$ versus dansyl-GCIIL (Danenberg & Cleland, 1975).

Induced substrate inhibition by dansyl-GCIIL should be competitive with respect to GGPP (G), since at a saturating concentration of GGPP all enzyme should be in the E•G form. Conversely, a noncompetitive pattern is expected if the substrate inhibition resulted from a different mechanism, such as dansyl-GCIIL binding to a secondary site on the enzyme. A double reciprocal plot in which GGPP was varied from 1 to 7 μM at three different inhibitory levels of dansyl-GCIIL (2.9, 5.7, and 8.5 μM) in the presence of 14 μM 1-P-GGOH gave a competitive inhibition pattern consistent with the mechanism presented in Scheme 2, although a small deviation from the expected competitive pattern was observed for the lowest concentration of dansyl-GCIIL, where substrate inhibition was weakest. Values of K_{is} (5 μM) and K_M^D (4 μM) were determined from a slope replot.

DISCUSSION

Previous steady-state kinetic studies with the mammalian form of PGGTase-I indicated that the enzyme binds substrates by a random sequential mechanism (Zhang et al., 1994b; Yokoyama et al., 1995). Isotope trapping experiments, however, suggested that an ordered binding of substrates was required to produce a competent ternary complex. Enzyme-bound GGPP was trapped as product before dissociating from the enzyme upon addition of a peptide substrate; whereas, enzyme-bound peptide was not trapped by GGPP (Yokoyama et al, 1995). These results were interpreted to suggest that PGGTase-I adds substrates by a "formally random," mechanism, while possessing a kinetically preferred pathway where GGPP binds before peptide (Yokoyama et al., 1995). Similar results were reported for mammalian PFTases (Pompliano et al., 1993; Furfine at al., 1995). However, a steady-state kinetic analysis of the yeast enzyme with dead-end inhibitors for FPP and peptide gave classic double reciprocal plots for an ordered mechanism where FPP adds first (Dolence et al., 1995).

PFTases, from a variety of sources (Dolence et al., 1995) and mammalian PGGTase-I (Yokoyama et al., 1995) are inhibited by their peptide substrates. Dolence and co-workers (1995) concluded for the yeast enzymes that inhibition probably resulted from nonproductive binding of the peptide in a manner that blocked entry of FPP into the catalytic site. Our earlier studies with yeast PGGTase-I gave no indication of substrate inhibition over a range of peptide concentrations, $0.5 \leq K_M \leq 5$ (Stirtan & Poulter, 1995). Since the amino acid sequences of PFTases and PGGTases have substantial similarity, indeed they have a common subunit in their host organisms, one anticipates similar binding mechanisms for the two enzymes. The absence of substrate inhibition for yeast PGGTase-I presumably resulted from a higher dissociation constant for the enzyme−peptide complex. However, when the yeast enzyme was incubated with 1-P-GGOH, an unreactive analog of GGPP, the peptide substrate became a potent inhibitor. These results are consistent with an ordered addition of substrates where binding of the second substrate, in this case peptide, is enhanced substantially by binding of the first.

The Michaelis and dissociation constants for GGPP with yeast PGGTase-I are significantly higher than previously reported for its mammalian counterpart (Zhang et al., 1994b; Yokoyama et al., 1995). A similar trend was found for PFTases (Cassidy et al., 1995; Dolence et al., 1995). The phenomenon has been reported for other prenyltransferases and may reflect intrinsic differences in the levels of intermediates in the isoprenoid pathways in yeast and mammals.

In contrast to mammalian PGGTase-I, the recombinant yeast enzyme gave double reciprocal plots with dead-end inhibitors that were fully consistent with an ordered mechanism. Cys-AMBA-Leu was competitive against dansyl-GCIIL and uncompetitive against GGPP. 1-P-GGOH gave a classic competitive profile against GGPP and induced substrate inhibition by dansyl-GCIIL in a manner consistent with the binding mechanism outlined in Scheme 2.

In summary, steady state kinetic studies of recombinant yeast PGGTase-I indicate that the enzyme binds substrates by an ordered mechanism with addition of GGPP before peptide. In contrast to other PFTases and PGGTases-I, the yeast enzyme is not inhibited by its peptide substrate at concentrations up to $5.0K_M$. However, strong substrate inhibition is seen in the presence of a dead-end inhibitor for GGPP, indicating a substantial synergistic effect in substrate binding. These results, taken together with the uncompetitive inhibition profile from our dead-end peptide substrate analog, firmly support an ordered mechanism.

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