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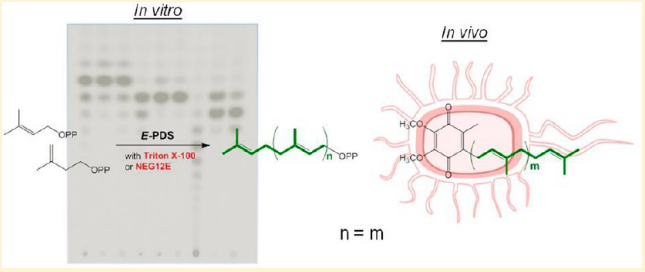
Dependence of the Product Chain-Length on Detergents for Long-Chain *E*-Polyprenyl Diphosphate Synthases

Jian-Jung Pan, Gurusankar Ramamoorthy, and C. Dale Poulter*

Department of Chemistry, University of Utah, 315 South 1400 East, Salt Lake City, Utah 84112, United States

Supporting Information

ABSTRACT: Long-chain *E*-polyprenyl diphosphate synthases (*E*-PDS) catalyze repetitive addition of isopentenyl diphosphate (IPP) to the growing prenyl chain of an allylic diphosphate. The polyprenyl diphosphate products are required for the biosynthesis of ubiquinones and menaquinones required for electron transport during oxidative phosphorylation to generate ATP. *In vitro*, the long-chain PDSs require addition of phospholipids or detergents to the assay buffer to enhance product release and maintain efficient turnover. During preliminary assays of product chain-length with anionic, zwitterionic, and nonionic detergents, we discovered considerable variability. Examination of a series of nonionic PEG detergents with several long-chain *E*-PDSs from different organisms revealed that *in vitro* incubations with nonaethylene glycol monododecyl ether or Triton X-100 typically gave chain-lengths that corresponded to those of the isoprenoid moieties in respiratory quinones synthesized *in vivo*. In contrast, incubations in buffer with *n*-butanol, CHAPS, DMSO, *n*-octyl-*β*-glucopyranoside, or *β*-cyclodextrin or in buffer without detergent typically proceeded more slowly and gave a broad range of chain-lengths.



Polyprenyl diphosphate synthases (PDS) catalyze 1′-4 chain elongation by the sequential addition of the growing isoprenoid chain in an allylic diphosphate to isopentenyl diphosphate (IPP) to generate linear allylic diphosphates with two or more isoprene units. The enzymes are selective for the ultimate chain-length of their products and the stereochemistry of the double bond, *E* or *Z*, introduced during each addition to IPP. The *E*-PDSs generally catalyze the formation of products with shorter chains, C_{10-50}. *Z*-PDSs typically give compounds with 11 (C_{55}) or more isoprene units including natural rubber, although a few instances of short-chain *Z*-PDSs have been reported.[1,2] The linear polyprenyl disphosphates are essential substrates for the biosynthesis of all but the simplest isoprenoid compounds found in nature. Farnesyl diphosphate (FPP, C_{15}) is required for synthesis of sterols, sesquiterpenes, and farnesylated proteins, while geranylgeranyl diphosphate (GGPP, C_{20}) is the precursor for carotenoids, diterpenes, and geranylgeranylated proteins. These metabolites perform numerous essential functions in cells and are a rich source for potential human therapeutics. The longer chain *E*-allylic diphosphates (typically $C_{30}-C_{50}$) are required for the biosynthesis of the isoprenoid side chain of ubiqunones (UQ) and menaquinones (MQ) in different species, such as UQ-6 in *Saccharomyces cerevisiae*, UQ-8 in *Escherichia coli*, and UQ-10 in humans. The respiratory quinones are intermediates in the electron transport in the cellular respiratory metabolism to generate ATP. *Z*-PDSs synthesize linear $C_{55}-C_{100+}$ isoprenoid molecules that serve as a lipid carrier during peptidoglycan biosynthesis of bacterial cell walls and glycoprotein biosynthesis in eukaryotes.

Purified long-chain PDSs require phospholipids or detergents for efficient turnover, presumably due to the slow release of the hydrophobic products. Their stimulatory effect appears to be enzyme-dependent.[3] For instance, Triton X-100 is a good activator for the *Lactobacillus plantarum*, *Micrococcus luteus*, and *Bacillus subtilis* undecaprenyl diphosphate (UPPS) synthases, while deoxycholate is only effective for the *L. plantarum* enzyme. The stimulatory effect of phospholipids also varies with enzyme and the type of phospholipids. In addition to stimulating activity, phospholipids and detergents can also affect the product profile. For example, the chain-length of the major product for *M. luteus* UPPS shifted from C_{50} to C_{55} when Triton X-100 was replaced by a phospholipid extract. Without a phospholipid or detergent in the assay buffer, *E. coli* UPPS slowly produced a series of products with chain-lengths up to C_{75}.[4] A microsomal preparation of dehydrodolichyl diphosphate synthase, a C_{90} and C_{95} *Z*-PDS from rat liver, gave shorter $C_{55}-C_{80}$ products with increasing concentrations of different detergents.[5]

Only a few studies have been reported for the effects of detergent and phospholipids on the activity and product distribution of long-chain *E*-PDSs. The activity of solanesyl diphosphate (SPP, C_{45}) synthase is stimulated by Tween-80, bovine serum albumin (BSA), and bacitracin. The *M. luteus* enzyme was also stimulated by a high molecular-mass fraction

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(HMF) from the bacterium. HMF is thought to bind to the C_{45}−PP products and presumably promotes turnover by providing a hydrophobic reservoir for the products.[6] In related studies with *E. coli* octaprenyl diphosphate (OPP, C_{40}) synthase, CHAPS and Triton X-100 were stimulatory, while Tween-80, deoxycholic acid, and *n*-octyl glucoside were not.[7] BSA and HMF were also activators. The effects of these reagents on product distribution were not reported, except that in the absence of Triton X-100 overelongated products (C_{45}−C_{60}) were found. Similar results were observed for *Paracoccus denitrificans* decaprenyl diphosphate (DPP, C_{50}) synthase, which gave a C_{65} product in the absence of Triton X-100,[8] and a *Cryptosporidium parvum* nonspecific PDS, where the chain-lengths of the products increased from C_{20}−C_{25} in the presence of Tween-20 to $\geq C_{40}$ in its absence.[9] We now report an investigation of the effect of several different detergents on the activities and product distributions as part of a study of the functions of long-chain *E*-PDSs.

MATERIALS AND METHODS

Reagents. All the chemicals were purchased from Sigma-Aldrich. [4-^{14}C]IPP was purchased from PerkinElmer. DMAPP, GPP, and FPP were synthesized according to that of Davisson et al.[10] Potato acid phosphatase was purchased from Roche.

Enzymes. All the enzymes were provided by the Enzyme Function Initiative (EFI) Protein Core at the Albert Einstein College of Medicine. The detailed procedures of the enzyme expression and purification are published elsewhere.[11]

Product Analysis. Incubations were carried out at 30 °C for 2 h in 40 μL of a mixture of 35 mM HEPES buffer, pH 7.6, containing 5 mM β-mercaptoethanol, 10 mM $MgCl_2$, and 200 μM [^{14}C]IPP and 50 μM allylic substrate (DMAPP, GPP, or FPP), unless otherwise specified. The reaction was quenched with 0.5 M EDTA, pH 8.0, extracted with 3 × 80 μL of water-saturated *n*-butanol. The butanol extract was transferred to an Eppendorf tube, solvent was removed on a SpeedVac, and 200 μL of 50 mM sodium acetate buffer, pH 4.7, containing 2 units of potato acid phosphatase (2 units/mg), 0.1% (v/v) Triton X-100, and 20% (v/v) *n*-propanol were added. The mixture was incubated overnight at 37 °C, and the isoprenoid alcohols were extracted with 3 × 200 μL of hexanes or methyl *t*-butyl ether (MTBE). The volume was concentrated on the SpeedVac and resuspended in 15 μL of hexanes or MTBE. Samples were spotted on RP-C18 TLC plates and developed with 19:1 (v/v) acetone/water. The developed TLC plates were visualized by phosphorimaging (Molecular Dynamics storage phosphor screen) using a Typhoon 8600 Variable Mode Imager (GE Healthcare).

Kinetic Studies. Initial velocities were measured by the acid-lability assay[12] for incubations of 400 μL samples of 35 mM HEPES buffer, pH 7.6, containing 5 mM β-mercaptoethanol, 10 mM $MgCl_2$, varied concentrations of [^{14}C]IPP, FPP, and detergents at 37 °C for 10 min. The reaction was initiated by addition of enzyme, and samples were removed periodically and immediately quenched with MeOH/HCl = 3:1, followed by incubation at 37 °C for 10 min. The sample was extracted with 400 μL of ligroin (bp 95−110 °C), and the radioactivity in a 200 μL portion was quantified by liquid scintillation spectrometry on a Tri-Carb 2910TR liquid scintillation analyzer (Perkin Elmer). The data were fit to the Michaelis−Menten equation using GraFit 5.0 (Erithacus Software).

RESULTS AND DISCUSSION

As part of a study to determine the function of isoprenoid chain elongation prenyltransferases identified in this work by their GI (GenInfo Identifier) designations, we examined a diverse group of putative chain elongation enzymes within a larger cluster of proteins in the isoprenoid synthase superfamily.[11] One of the challenges in the study was the selection of a buffer system that gave maximal activity and a set of products whose chain-lengths most closely approximated those formed in vivo. Since examination of previous reports gave no clear consensus for which set of reaction conditions were optimal, the chain-length selectivity of several *E*-PDSs for a series of reagents that could alter the solubility of the hydrophobic long-chain isoprenyl diphosphate products was studied.

Effect of Buffer Composition on Product Distributions. Product distributions used for the PDSs were studied in HEPES buffer supplemented with the following reagents: *n*-butanol, dimethyl sulfoxide (DMSO), sodium dodecyl sulfate (SDS), CHAPS, β-cyclodextrin (CD), 3,6,9-heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (MCD), *n*-octyl-β-glucopyranoside (OGP), Triton X-100 (TX), pentaethylene glycol monododecyl ether (PEG12E), hexaethylene glycol monododecyl ether (HEG12E), octaethylene glycol monododecyl ether (OEG12E), nonaethylene glycol monododecyl ether (NEG12E), and octaethylene glycol tetradecyl ether (OEG14E). Incubations with enzyme GI 19551716 in buffer containing 5−25% (v/v) *n*-butanol gave little turnover, resulting in trace amounts of products, perhaps due to denaturation of the enzymes (supplementary data, see SI, TLC1). Similar results were obtained with several enzymes in the presence of 0.5 mM SDS (supplementary data, see SI, TLC2). In the presence of DMSO or OGP, the PDSs were less selective and gave longer chain-lengths than for incubations in 0.1% (1.6 mM) TX (see SI, TLCs 1,3,4). Product distributions in buffer containing CHAPS were dependent on detergent concentration, and are compared with 0.1% TX in Figure 1A,B. The predominant product for enzyme GI 19551716 is a C_{45} allylic diphosphate in buffer with TX, accompanied by smaller amounts of C_{40} and C_{50} products. The C_{45} diphosphate also

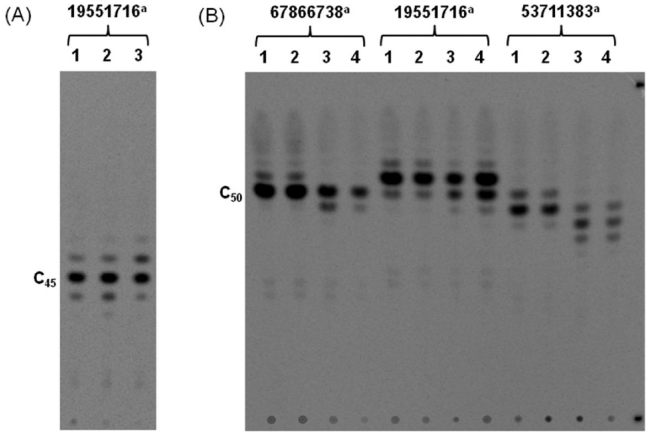

Figure 1. Product distributions for GI 19551716, GI 67866738, and GI 53711383 for 2 h incubations in buffer containing TX or CHAPS. (A) Production distributions for GI 19551716 in buffer containing 0.1% TX (lane 1), 2.5 mM CHAPS (lane 2), 5.0 mM CHAPS (lane 3). (B) Production distributions for GI 19551716, GI 67866738, and GI 53711383 in buffer containing 0.1% TX (lane 1), 0.05% TX (lane 2), 0.01% TX (lane 3), 2.0 mM CHAPS (lane 4). [a]C-terminal His_6 tag.

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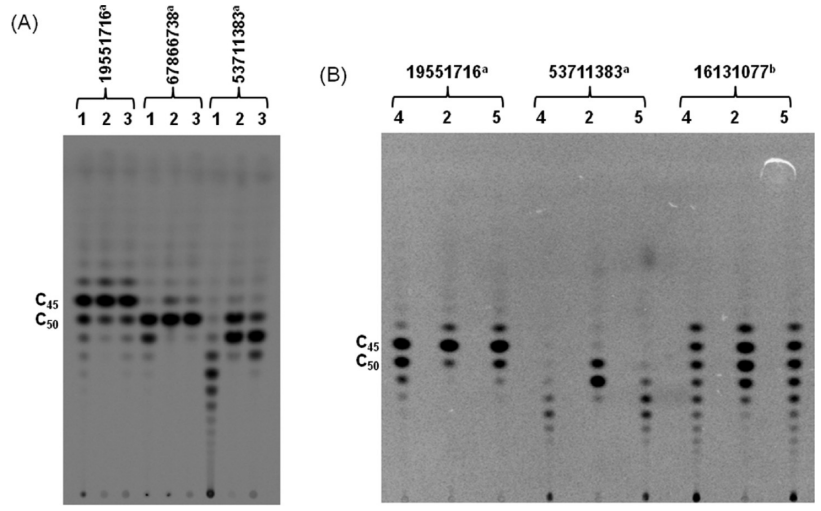

Figure 2. Product distributions of GI 16131077, GI 19551716, GI 53711383, and GI 67866738 for 2 h incubations in TX (0.1%, lane 2). Part A; CD (1.6 mM, lane 1), MCD (2 mM, lane 3); Part B; CD (0.2 mM, lane 4), MCD (0.2 mM, lane 5). [a]C-terminal His_6 tag. [b]N-terminal His_6 tag.

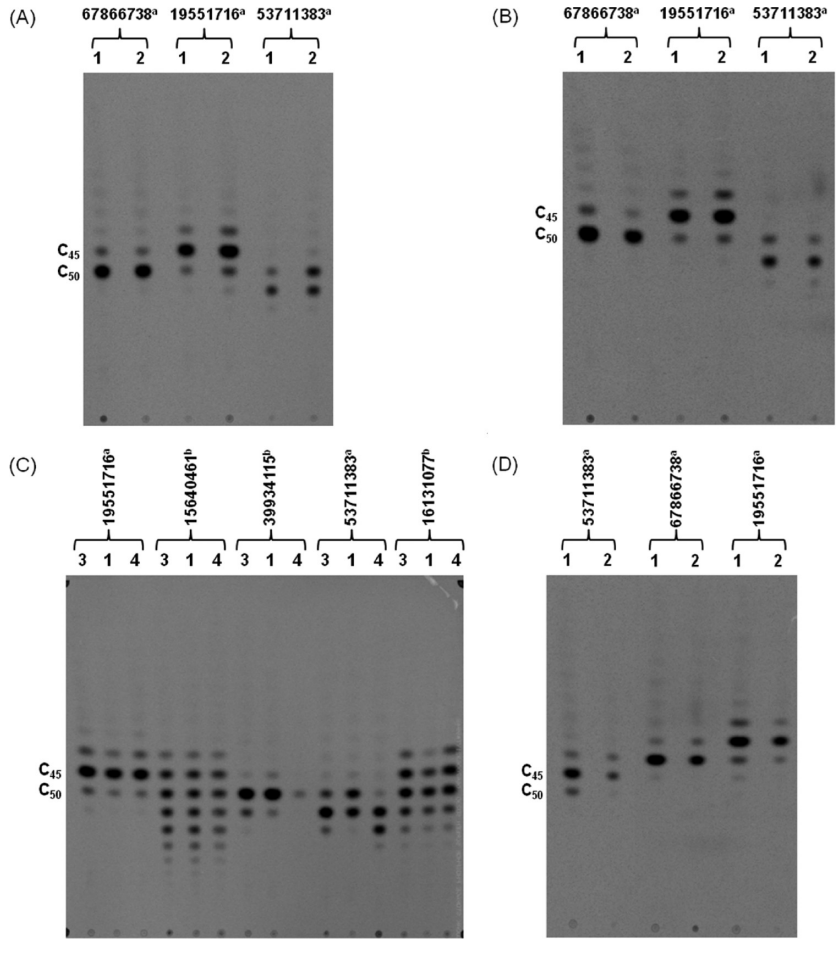

Figure 3. Product distributions for GI 15640461, GI 16131077, GI 19551716, GI 39934115, GI 53711383, and GI 67866738 for 2 h incubations. Part A; lane 1, 0.1% TX; lane 2, 2 mM PEG12E; Part B; lane 1, 0.1% TX; lane 2, 2 mM NEG12E; Part C; lane 1, 0.1% TX; lane 3, 2 mM HEG12E; lane 4, 2 mM OEG12E; Part D; lane 1, 0.1% TX; lane 2; 2 mM OEG14E. [a]C-terminal His_6 tag. [b]N-terminal His_6 tag.

predominates in the presence of CHAPS; however, the amount of C_{50} material increases slightly at 2.5 mM and the amount of C_{40} product increases in 5 mM relative to TX (Figure 1A). As the concentration of TX was reduced from 0.1% to 0.01%, the product distribution shifted to longer chain-lengths, as seen for GI 19551716, GI 53711383, and GI 67866738, where the profiles are similar at 0.1% and 0.05% but shift substantially at 0.01% (Figure 1B). Product distributions in 2 mM CHAPS are similar to those for 0.01% TX. The optimal concentration of TX for selective production of products appears to be 0.05–0.1%.

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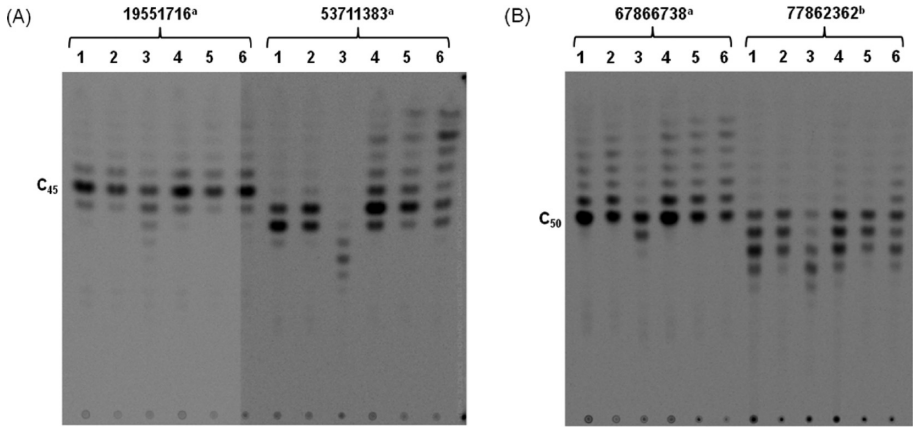

Figure 4. Product distributions 2 h incubations with different ratios of IPP:DMAPP and IPP:FPP. Lane 1, IPP:DMAPP = 4:1, 0.1 % TX; Lane 2, IPP:DMAPP = 1:2, 0.1 % TX; Lane 3, IPP:DMAPP = 1:2, no TX; Lane 4, IPP:FPP = 4:1, 0.1 % TX; Lane 5, IPP:FPP = 1:2, 0.1 % TX; Lane 6, IPP:FPP = 1:2, no TX. Part A; GI 1955176, GI 53711383; Part B; GI 67866738, and GI 77862362. [a]C-terminal His_6 tag. [b]N-terminal His_6 tag.

Considerable variation was seen in chain-lengths for different PDSs in buffers containing CD or MCD relative to 0.1% TX. At 2 mM MCD (Figure 2A), the product distributions for GI 19551716 (C_{45}), GI 53711383 (C_{55}), and GI 67866738 (C_{50}) were similar to those for 0.1% Triton X-100. Buffer with 1.6 mM CD gave a slight increase in higher chain-length products for GI 19551716. A more substantial increase in chain-length (C_{50} to C_{55}) was seen for GI 67866738, while GI 53711383 gave a substantial shift to higher chain-lengths with C_{65} allylic diphosphate as the major product along with substantial amounts of C_{60}–C_{75} chains. The profiles for GI 19551716 were similar for buffers containing 0.2 mM CD and MCD (Figure 2B), while for GI 53711383, longer chain products were produced less selectively. GI 16131077, which appears to be inherently less selective in 0.1% TX buffer than the other three enzymes listed in Figure 2 (compare lanes 2), gave an even broader distribution of products in 0.2 mM CD and MCD buffers.

The PEG-based nonionic detergent TX consistently gave the narrowest distribution of chain-lengths. However, TX is a mixture consisting of a 4-(1,1,3,3-tetramethylbutyl)phenyl moiety attached to PEG units of different lengths. This heterogeneity sometimes complicates the isolation and purification of the products. We compared 2 mM concentrations of a series of dodecyl ether PEG detergents containing PEG_5 (PEG12E), PEG_6 (HEG12E), PEG_8 (OEG12E), and PEG_9 (NEG12E) units and a PEG_8 tetradecyl ether (OEG14E) with 0.1% TX to examine the effect of the nonionic detergents on the length of the hydrocarbon chain (Figure 3). While all of the alkoxy PEG detergents gave narrow product distributions, the profiles for NEG12E most closely matched those of TX and, in one case (see GI 678666738 in Figure 3B), was more selective.

The long-chain *E*-PDSs typically prefer DMAPP or FPP as the initial allylic substrate, and the IPP/allylic substrate ratio can affect product distributions.[13−16] One might expect that the longest chain product would depend strongly on the initial IPP/allylic diphosphate ratio; for example, IPP/DMAPP = 4:1 would give mostly the C_{25} diphosphate. However, long-chain PDSs selectively elongate by binding the intermediates more tightly than the starting substrates and terminate when the growing chain fills the elongation cavity.[4,7] Over elongation is seen under forcing conditions, presumably from a change in conformation of the enzyme induced by the isoprenoid chain to create a longer binding channel.[11] Figure 4 shows the product distributions for four enzymes and different ratios of DMAPP and FPP. The product distributions were similar in 0.1% TX buffer for DMAPP and FPP at 4:1 and 1:2 ratios of IPP/allylic substrate. In buffer without TX, the reactions were slower, less selective, and somewhat overelongated, even when the IPP/allylic ratio was 1:2.

Additional comparisons are shown in Figure 5. Over elongation was seen for the allylic substrates FPP and geranyl

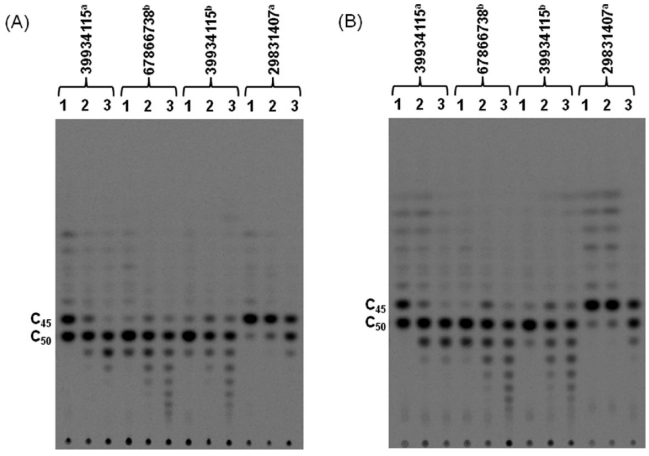

Figure 5. Product distributions for GI 29831407, GI 39934115, and 67866738 for 2 h incubations in the presence and absence of TX. Part A; IPP/allylic diphosphate = 4:1. Lane 1, FPP, 0.1% TX; Lane 2, FPP, no TX; Lane 3, DMAPP, no TX. Part B; IPP/allylic diphosphate = 4:1. Lane 1, GPP, 0.1% TX; Lane 2, GPP, no TX; Lane 3, DMAPP, no TX. [a.] C-terminal His_6 tag. [b.] N-terminal His_6 tag.

diphosphate (C_{10}, GPP) in the absence of TX, but both gave a narrower range of chain-lengths than DMAPP. This observation suggests that the longer allylic substrates, FPP and GPP, help promote chain-length selectivity by competing better than DMAPP with longer chain-length products for binding to the enzymes. Interestingly, the product distributions at 20:1 ratios of IPP/FPP or DMAPP in 24 h incubations (Figure 6) were similar to those determined for 2 h incubations with 4:1 IPP and allylic substrates. The product distributions for two enzymes, GI 29376566 and GI 58578715, were similar with, or without, TX in the buffer. It is possible that these enzymes

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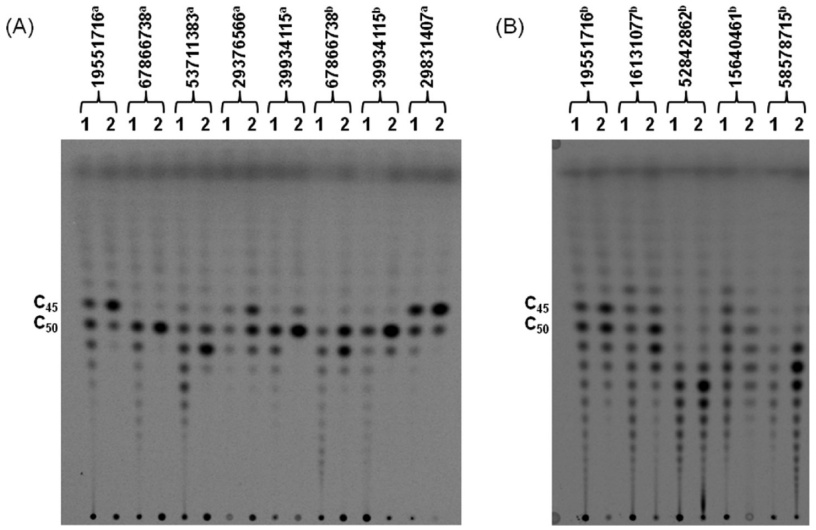

Figure 6. Product distributions for 24 h incubations in the presence and absence of TX where IPP/FPP = 20:1. Part A. GI 19551716, GI 29376566, GI 29831407, GI 39934115, 53711383, and GI 67866738; Part B, GI 15640461, GI 16131077, GI 19551716, GI 52842862, and GI 58578715. Lane 1, no TX; Lane 2, 0.1% TX. [a]C-terminal His_6 tag. [b]N-terminal His_6 tag.

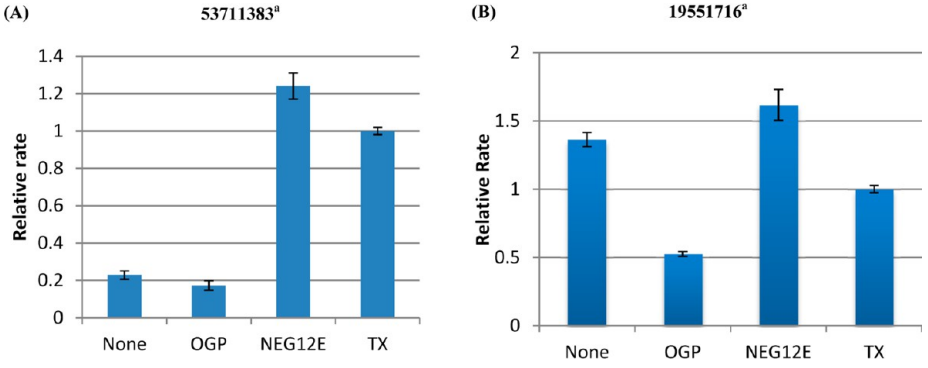

Figure 7. Relative chain elongation rates for GI 53711383 (Part A) and GI 19551716 (Part B). Buffers contained 50 μM IPP, 5 μM FPP, and no detergent, 2 mM OGP, 2 mM NEG12E, or 0.1% TX. [a]C-terminal His_6 tag.

are normally associated with another protein *in vivo*, that facilitates product release.[6] In addition, it is also important to note the influence that a His_6 tag can exert on the distributions of products. Those shown in Figures 3A,B,D and 5A,B for GI 67866738, a C_{50} elongation enzyme, are similar for C-terminal His_6 tagged, and N-terminal His_6 tagged versions of the protein. In contrast, N-terminal His_6 tagged GI 39934115 are C_{50} elongation enzymes, while the C-terminal His_6 tagged protein gives approximately equal amounts of C_{45} and C_{50} products (Figures 3C and 5A,B). A much larger difference is seen for GI 19551716, where the C-terminal tagged proteins synthesize C_{45} products, while the N-terminally tagged enzyme gives a broad distribution of products between C_{45} and C_{70} (Figures 3C and 6A,B). Thus, it is advisable to work with tagless or both N- and C-terminal tag versions of the recombinant proteins when determining function.

Effects of Detergents on Kinetic Parameters. Steady-state kinetic constants were determined for PDSs GI 53711383 and GI 19551716 in buffer containing 0.1% TX. For GI 53711383, k_{cat} = 0.03 s^{-1}, K_M^{IPP} = 11 μM, and K_M^{FPP} = 0.05 μM and for GI 19551716, k_{cat} = 0.01 s^{-1}, K_M^{IPP} = 13 μM, and K_M^{FPP} = 0.28 μM. Relative rates were then measured for both enzymes in buffers containing saturating concentrations of IPP (50 μM) and FPP (5 μM) and no detergent or 2 mM OGP, 2 mM NEG12E, or 0.1% TX. For GI 53711383, ~10-fold stimulations of the rate for chain elongation were seen for NEG12E and TX (Figure 7A), while the rate in buffer containing OGP is similar to that in the absence of a detergent. The enzyme gave a tight distribution consisting of C_{50} and C_{55} products in TX and NEG12E buffers, and a broad distribution between C_{30} and C_{65} in OGP or without detergent (Figure 8A). Detergents had a much smaller effect on the rate of chain elongation for GI 19551716 (Figure 7B) with a slight increase for NEG12E and slight decreases for OGP and TX. Tight product distributions with a maximum at C_{45} were seen for NEG12E and TX. A slight increase in the amount of C_{50} diphosphate was seen with OGP (Figure 8B). A broader distribution between C_{45} and C_{60} was seen without detergent. Previously, small 3-fold rate enhancements were reported for the *E. coli* C_{40} synthase in TX.[7] The rate enhancement was not large enough to switch the rate-determining step from the product release to the chemical steps,[7] and the authors suggested that another mechanism for enhancing product release/sequestration might operate *in vivo*.

CONCLUSIONS

As chain-length increases, the polyisoprenoid diphosphate products of chain elongation reactions become increasingly hydrophobic. *In vivo*, the longer chain-length compounds can affiliate with membrane, bind to and be transported by proteins,

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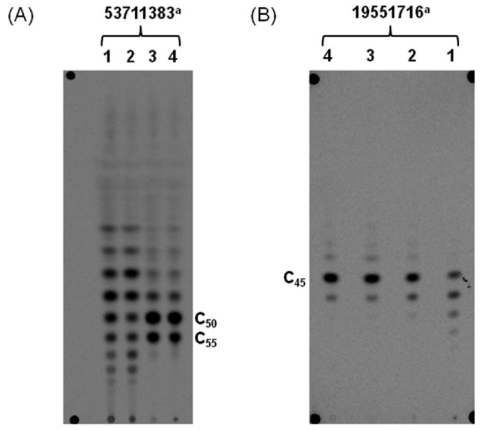

Figure 8. Product distributions for GI 53711383 (Part A) and GI 19551716 (Part B). Buffers contained 50 μM IPP, 5 μM FPP, and none (lane 1), 2 mM OGP (lane 2), 2 mM NEG12E (lane 3), or 0.1% TX (lane 4). [a]C-terminal His_6 tag.

and converted to downstream metabolites. All of these scenarios remove the products from the vicinity of the enzyme and promote turnover. *In vitro*, the hydrophobic products accumulate and can bind to the enzyme, thereby slowing the chain elongation process by product inhibition and serve as substrates for further prenyltransfers to produce "over-elongated" products. Inclusion of detergents in the assay buffer typically promotes turnover and selectivity.

We and others found that Triton X-100 (TX) consistently stimulates activity and enhances selectivity.[13,16,17] In addition, we found that the chain-lengths of polyisoprenoid diphosphates generated by *E*-PDSs in TX buffer are similar to those found in metabolites containing the polyisoprenoid chains synthesized *in vivo*. As seen in Table 1, the principal products we detected for nine long-chain *E*-PDSs in the presence of TX closely matched the chain-lengths of the polyisoprenoid chains found in menaquinone and ubiquinones isolated from the corresponding organisms. TX may facilitate the rate and selectivity of chain elongation by solubilizing the hydrophobic products and competing with polyisoprenoid products for binding to the active site of the chain elongation enzymes. A crystal structure of undecaprenyl diphosphate synthase shows two molecules of TX in the active site tunnel.[18] Detergent binding is consistent with the observation that activity is reduced to levels seen in its absence, while selectivity is retained at high concentrations (>1%, 16 mM).

Finally, Triton X-100 is a good detergent for *in vitro* kinetic and product studies of *E*-PDSs. However, TX is a mixture of compounds where the hydrophobic (tetramethylbutyl)phenyl moiety is attached to a series PEG units of different lengths consisting of an average 9.5 ethylene glycol monomers. In some applications, the mixture of detergent molecules complicates the isolation and purification of products from the enzyme-catalyzed reactions. As a result, we were interested in finding a monomeric nonionic detergent that would minimize this problem and examined a series of *n*-alkyl PEG ethers. Preliminary experiments indicated that the lengths of the hydrocarbon and PEG regions of the detergents were important. Of the PEGs studied, we found that NEG12E consistently gave polyprenyl diphosphates with the narrowest product distributions and the chain-lengths corresponded to those synthesized *in vivo* and to the chain-lengths found for TX. Clearly care should be taken in the selection of detergents for *in vitro* studies of the long-chain elongation enzymes, and NEG12E and TX are good choices for a wide variety of *E*-PDSs.

■ ASSOCIATED CONTENT

Supporting Information

TLCs 1−4 with *n*-butanol, SDS, DMSO, and OGP; Michaelis−Menten plots for GIs 53711383 and 19551716; TLCs with different allylic substrates, DMAPP, and FPP (adapted from ref 11). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: (801) 581-6685. E-mail: poulter@chem.utah.edu.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

PDS: polyprenyl diphosphate synthases; DMAPP: dimethylallyl diphosphate; IPP: isopentenyl diphosphate; ATP: adenosine

Table 1. Comparison of in Vitro and in Vivo Chain-Lengths for *E*-PDSs

GI	annotation [organism]	in vitro[a]	in vivo[b]	refs
67866738	decaprenyl diphosphate synthase [*Agrobacterium tumefaciens*]	C_{50}	C_{50} (UQ-10)	26
19551716	geranylgeranyl pyrophosphate synthase [*Corynebacterium glutamicum* ATCC 13032]	C_{45}	C_{45} (MK-9)	20
53711383	octaprenyl-diphosphate synthase [*Bacteroides fragilis* YCH46]	C_{50}/C_{55}	C_{50}/C_{55} (MK-10/11)	25
39934115	farnesyltranstransferase [*Rhodopseudomonas palustris* CGA009]	C_{50}	C_{50} (UQ-10)	23
29831407	polyprenyl diphosphate synthase [*Streptomyces avermitilis* MA-4680]	C_{45}	C_{45} (MK-9)	22
29376566	heptaprenyl diphosphate synthase, component II, putative [*Enterococcus faecalis* V583]	C_{45}	C_{45} (DMK-9)	21
16131077	octaprenyl diphosphate synthase [*Escherichia coli* str. K-12 substr. MG1655]	C_{45}−C_{55}	C_{40} (UQ-8)	19
77862362	decaprenyl diphosphate synthase [*Rhodobacter capsulatus*]	C_{50}-C_{60}	C_{50} (UQ-10)	27
52842862	octaprenyl diphosphate synthase IspB [*Legionella pneumophila* subsp. pneumophila str. Philadelphia 1]	C_{60}-C_{70}	C_{55}−C_{65} (UQ-11/12/13)	24
58578715	octaprenyl-diphosphate synthase [*Ehrlichia ruminantium* str. Welgevonden]	C_{55}−C_{65}	NK	
15640461	octaprenyl-diphosphate synthase [*Vibrio cholerae* O1 biovar El Tor str. N16961]	C_{45}−C_{60}	NK	

[a]With TX. [b]UQ: ubiquinone. MK: menaquinone. DMK: demethylmenaquinone. NK: not known.

triphosphate; PEG: polyethylene glycol; DMSO: dimethyl sulfoxide; GPP: geranyl diphosphate; FPP: farnesyl diphosphate; GGPP: geranylgeranyl diphosphate; OPP: octaprenyl diphosphate; SPP: solanesyl diphosphate; DPP: decaprenyl diphosphate; UPP: undecaprenyl diphosphate; UQ: ubiquinone; MQ: menaquinone; BSA: bovine serum albumin; HMF: high molecular-mass fraction; CHAPS: 3-[(3-cholamidopropyl)dimethylamino]-1-propanesulfonate; MTBE: methyl *t*-butyl ether; TLC: thin-layer chromatography; HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS: sodium dodecyl sulfate; CD: *β*-cyclodextrin; MCD: 3,6,9-heptakis-(2,6-di-*O*-methyl)-*β*-cyclodextrin; OGP: *n*-octyl-*β*-glucopyranoside; TX: Triton X-100; PEG12E: pentaethylene glycol monododecyl ether; HEG12E: hexaethylene glycol monododecyl ether; OEG12E: octaethylene glycol monododecyl ether; NEG12E: nonaethylene glycol monododecyl ether; OEG14E: octaethylene glycol tetradecyl ether

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