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Mutagenesis of Isopentenyl Phosphate Kinase to Enhance Geranyl Phosphate Kinase Activity

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Abstract

Isopentenyl phosphate kinase (IPK) catalyzes the ATP-dependent phosphorylation of isopentenyl phosphate (IP) to form isopentenyl diphosphate (IPP) during biosynthesis of isoprenoid metabolites in Archaea. The structure of IPK from the archaeon *Thermoplasma acidophilum* (THA) was recently reported and guided the reconstruction of the IP binding site to accommodate the longer chain isoprenoid monophosphates geranyl phosphate (GP) and farnesyl phosphate (FP). We created four mutants of THA IPK with different combinations of alanine substitutions for Tyr70, Val73, Val130 and Ile140, amino acids with bulky side chains that limited the size of the side chain of the isoprenoid phosphate substrate that could be accommodated in the active site. The mutants had substantially increased GP kinase activity, with 20 to 200-fold increases in k_{cat}^{GP} and 30–130-fold increases in k_{cat}^{GP}/K_M^{GP} relative to that of wild type THA IPK. The mutations also resulted in a 10⁶-fold drop in k_{cat}^{IP}/K_M^{IP} compared to wild-type IPK. No significant change in the kinetic parameters for the cosubstrate ATP were observed, signifying that binding between the nucleotide binding site and the IP binding site was not cooperative. The shift in substrate selectivity from IP to GP, and to a lesser extent, FP, in the mutants could act as a starting point for the creation of more efficient GP or FP kinases whose products could be exploited for the chemoenzymatic synthesis of radiolabeled isoprenoid diphosphates.

Isopentenyl phosphate kinase (IPK) is a recently identified enzyme in the modified mevalonate (MVA) pathway in Archaea that catalyzes the ATP-dependent phosphorylation of isopentenyl phosphate (IP) to produce isopentenyl diphosphate (IPP), one of two building blocks for the biosynthesis of isoprenoid compounds (1). IPK and a putative phosphomevalonate decarboxylase are thought to complement the absence of phosphomevalonate kinase and diphosphomevalonate decarboxylase in the archaeal MVA pathway. This is accomplished by the conversion of phosphomevalonate to IPP via two consecutive steps that are the reverse of those found in the classical MVA pathway (Figure 1). As in the classic eukaryotic MVA pathway and the deoxyxylulose phosphate (DXP) pathway found in bacteria and plant chloroplasts, the IPP produced by the archaeal MVA pathway undergoes condensation reactions catalyzed by prenyltransferases to produce the vast number of isoprenoid compounds necessary to sustain life (2–5).

The crystal structures of IPK from three archaeal organisms have been determined (6,7). In particular, the structure of IPK from *Methanocaldococcus jannaschii* (MJ) guided the

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creation of mutants with observed kinase activities for the 15-carbon isoprenoid farnesyl phosphate (FP) (7). These variants contained different combinations of mutations located in the IP binding site that gave rise to their ability to bind and phosphorylate FP to form farnesyl diphosphate (FPP). While production of FPP by these mutants was confirmed using a coupled IPK–sesquiterpene synthase assay and GC–MS (8), the mutants were not characterized kinetically, and kinase activities for the 10-carbon isoprenoid, geranyl phosphate (GP) were not reported.

In this paper, we describe mutants of IPK from *Thermoplasma acidophilum* (THA) with significant GP kinase (GPK) activity, as well as weaker FP kinase (FPK) activity. In addition, these mutants have significantly lower IP kinase activity, indicating the successful conversion of IPK to GPK by structure-based engineering. Our work thus constitutes the first demonstration of a viable GPK enzyme with measurable FPK activity, which can be used in the chemoenzymatic synthesis of radiolabeled isoprenoid chains with potential application in research involving isoprenoid synthases and prenyltransferases (7).

Results and Discussion

Mutagenesis of THA IPK

Four mutants were created that contained different combinations of alanine substitutions for residues Tyr70, Val73, Val130 and Ile140. Tyr70 and Val73 are found on the long α C helix, and Val130 and Ile140 are on the β 9 and β 10 strands, respectively (Figure 2) (6). These residues are located at the distal end of the binding pocket for the isopentenyl moiety in IP. Val73, Val130 and Ile140 are near the bottom of the pocket, and based on inspection of the structure, mutation of these residues appeared to be necessary to enlarge the pocket in order to bind a geranyl unit. Inspection also suggested that the Y70A mutation is needed to accommodate a fully extended farnesyl unit. Table 1 lists the mutations contained in each IPK variant. Mutants YV₇₃V₁₃₀I, YV₇₃I, and YV₁₃₀I contain the Y70A mutation. V₇₃V₁₃₀I does not contain Y70A and was expected to have minimal FP kinase activity. YV₇₃V₁₃₀I also contains all of the different combinations of V73A, V130A and I140A found in the other three variants. Each mutant was purified to homogeneity (Figure 3a), and its ability to phosphorylate GP and FP was determined by autoradiography using [γ -³²P] ATP (Figure 3b). All four mutants exhibited strong GP kinase activity, as shown by an intense spot corresponding to GPP (R_f = 0.52). In addition, YV₇₃V₁₃₀I, YV₇₃I, and YV₁₃₀I, each containing the Y70A mutation, showed detectable FP kinase activity (a less intense spot, R_f = 0.75), while V₇₃V₁₃₀I without the Y70A mutation did not. Phosphorylation of GP appeared to be favored over FP or IP. These results indicate that the proteins fold into catalytically competent structures.

Kinetic Studies

GP and FP kinase activities of YV₇₃V₁₃₀I, YV₇₃I, YV₁₃₀I and V₇₃V₁₃₀I were measured using the coupled fluorescence assay by Pilloff *et al.* (9,10) and the results are summarized in Table 2. Values of k_{cat} and K_M for GP, FP and IP were measured at a saturating concentration of ATP (250 μ M).

Mutant YV₇₃V₁₃₀I

YV₇₃V₁₃₀I exhibited improved GP kinase activity compared to wild-type IPK. For this mutant, k_{cat} = 1.1 ($\pm$ 0.1) s⁻¹ represents a 22-fold increase relative to the GP kinase activity for wild-type THA IPK (10). In addition, K_M^{GP} = 2.4 ($\pm$ 0.3) $\times$ 10³ μ M for YV₇₃V₁₃₀I, a slight change from K_M^{GP} for the wild-type enzyme. The resulting catalytic efficiency is almost 50-fold larger than that for the wild-type enzyme. The k_{cat}^{FP} for phosphorylation by this mutant is two times lower than k_{cat}^{GP} for the same enzyme. Since the GP kinase activity

of wild-type THA IPK was very low, it was presumed that its promiscuous FP kinase activity would be negligible in comparison. The catalytic efficiency of YV₇₃V₁₃₀I for the phosphorylation of FP is comparable to that for phosphorylation of GP. This mutant also exhibited a measurable yet weak residual IP kinase activity which is 3100-fold lower than that of wild-type THA IPK. Moreover, K_M^{IP} of YV₇₃V₁₃₀I is 1200-fold higher than that of wild-type THA IPK, resulting in a 10⁶-fold decrease in k_{cat}^{IP}/K_M^{IP} . The k_{cat}^{ATP} and K_M^{ATP} for YV₇₃V₁₃₀I using GP and FP, and IP as cosubstrates are similar to those for wild-type THA IPK (Supporting Information). The Michaelis–Menten curves for YV₇₃V₁₃₀I (and other mutants), using GP, FP, or IP and ATP as cosubstrates are found in the Supporting Information.

Mutant YV₇₃I

YV₇₃I exhibited improved GP kinase activity compared to YV₇₃V₁₃₀I, with $k_{cat}^{GP} = 4.1 (\pm 0.2) \text{ s}^{-1}$ that is 90-fold greater than k_{cat}^{GP} for wild-type THA IPK, and just half of k_{cat}^{IP} for wild-type THA IPK. The Michaelis constant, $K_M^{GP} = 3.5 (\pm 0.3) \times 10^3 \mu\text{M}$, is similar to those for YV₇₃V₁₃₀I and wild-type THA IPK, resulting in a catalytic efficiency for GP kinase activity that is 3-fold and 120-fold greater than those of YV₇₃V₁₃₀I and wild-type THA IPK, respectively. This GP kinase efficiency is only 10³-fold lower than that of the native IP kinase activity of THA IPK and represents a significant activity for GP relative to native enzyme. The FP kinase activity of YV₇₃I is similar to that of YV₇₃V₁₃₀I, and its catalytic efficiency is only slightly lower than that of YV₇₃V₁₃₀I. The residual IP kinase activity of YV₇₃I is slightly higher than that of YV₇₃V₁₃₀I, and its catalytic efficiency is 10⁶-fold lower than of wild-type THA IPK.

Similar to the profile observed for YV₇₃V₁₃₀I, the mutations in the IP binding site did not affect the kinetic parameters for ATP in the presence of the three different isoprenoid phosphate substrates. The k_{cat} s and K_M s calculated for changes in [ATP] for YV₇₃I are similar to those measured for YV₇₃V₁₃₀I, the other two mutants YV₁₃₀I and V₇₃V₁₃₀I, and native THA IPK.

Mutant YV₁₃₀I

Among all four mutants tested, YV₁₃₀I had the highest k_{cat}^{GP} ($10.1 (\pm 0.7) \text{ s}^{-1}$), similar to k_{cat}^{IP} for wild-type THA IPK and more than twice the activity of YV₇₃I for GP. This is also more than 200-fold greater than the promiscuous activity of wild-type THA IPK for GP. $K_M^{GP} = 8.0 (\pm 1.1) \times 10^3 \mu\text{M}$ for YV₁₃₀I, leading to a catalytic efficiency that is only 10³-fold lower than that for wild-type THA IPK for its native substrate, IP. The turnover number and Michaelis constant for FP by YV₁₃₀I are similar to those for YV₇₃I. Finally, the remnant IP kinase activity is also similar to those of the previous mutants, with a low k_{cat}^{IP} that is almost 2000-fold lower than that for wild-type IPK. K_M^{IP} for YV₁₃₀I is also 2000-fold higher than that for wild-type THA IPK but similar to those measured for YV₇₃V₁₃₀I and YV₇₃I.

Mutant V₇₃V₁₃₀I

Among the four mutants, V₇₃V₁₃₀I did not contain the Y70A mutation, resulting in a smaller substrate-binding site that was presumably unable to bind FP unless the hydrocarbon chain in a more compact conformation. Modeling studies suggested that the active site of this mutant is sufficiently large to bind a fully extended GP. On the other hand, synthesis of FPP was not detected for V₇₃V₁₃₀I in assays using [γ -³²P] ATP (Figure 3b). $k_{cat}^{GP} = 2.8 (\pm 0.5) \text{ s}^{-1}$ for V₇₃V₁₃₀I reflects a 60-fold improvement in turnover number relative to wild-type THA IPK. $K_M^{GP} = 9.5 (\pm 0.3) \times 10^3 \mu\text{M}$, resulting in a 30-fold increase in catalytic efficiency for the phosphorylation of GP relative to the wild-type enzyme. The k_{cat} for V₇₃V₁₃₀I for IP is almost 10³-fold lower than that for wild-type THA

IPK, and K_M^{IP} is significantly higher than that for wild-type THA IPK. These result in a catalytic efficiency for the phosphorylation of IP by V₇₃V₁₃₀I that is more than 70,000-fold less than that for wild-type THA IPK.

UPLC–MS detection of GPP and FPP products of IPK Mutants

The ability of each IPK mutant to produce the isoprenoid diphosphate products from their respective isoprenoid monophosphate substrates was confirmed by negative ion UPLC–MS using a C18 column. The chromatograms for synthesis of GPP gave peaks at 1.11 min for GPP (m/z 313, C₁₀H₁₉ P₂O₇[−]) and at 3.39 min for GP (m/z 233, C₁₀H₁₈PO₄[−]), while those for synthesis of FPP gave peaks at 4.74 min for FPP (m/z 381, C₁₅H₂₇P₂O₇[−]) and 5.43 min for FP (m/z 301, C₁₅H₂₆O₄P[−]). IPP and IP could not be separated on C₁₈ or C₄ columns but masses for the negative ion forms of IPP (m/z 245, C₅H₁₁P₂O₇[−]) and IP (m/z 165, C₅H₁₀P[−]O₃) were detected in the void volume (see Supporting Information).

IPK Mutants with Triple Isoprenoid Monophosphate Kinase Activities

A total of four THA IPK mutants were created by structure-based redesign of the THA IPK active site by replacing bulky amino acids in the IP binding site with alanine (6). The IPK variants phosphorylate GP and FP, isoprenoid chains that are longer than IP. These enzymes should be useful for the synthesis of β-³²P labeled isoprenoid diphosphates and related molecules. The mutant kinases might also be useful *in vivo* to recycle isoprenoid monophosphates formed by hydrolysis of the corresponding diphosphates (7, 11–12).

Expansion of the IP binding site was based on the 2.0 Å crystal structure of THA IPK (6). For all of the mutants, K_M^{IP} increased ~10³-fold, while k_{cat}^{IP} decreased by a similar magnitude. The increase in K_M^{IP} might have resulted from the increase in size of the IP binding site that would allow IP to bind in a variety of unproductive conformations or have unfavorable interactions with water molecules in the enlarged active site. In contrast, modest changes in K_M^{GP} were observed in the THA IPK mutants relative to wild-type THA IPK, while turnover of GP increased 23–214-fold. Thus, the expansion of the hydrocarbon pocket in the active site allowed GP to bind in an orientation that facilitated phosphorylation.

Figure 4a shows a stable binding mode of GP in the YV₁₃₀I active site determined by molecular dynamics calculations, where it appears that the alanine mutations created an expanded binding site sufficient to bind a kinked conformer of GP. Among the enzymes studied, YV₁₃₀I exhibited the highest k_{cat}^{GP} , which is comparable to k_{cat}^{IP} of the wild-type THA IPK (10). The other mutants had slightly lower GP kinase activities. Analysis of the products by UPLC–MS showed a >80% conversion to products at substrate concentrations of 2 mM GP and 5 mM ATP. It was observed that the three mutants with the highest GP kinase activities (YV₁₃₀I, YV₇₃I, and V₇₃V₁₃₀I) had smaller substrate binding pockets than YV₇₃V₁₃₀I and suggested that a larger cavity did not necessarily translate to higher activity. It was also observed that the mutants containing the Y70A mutation had FP kinase activities that were weaker than their corresponding GP kinase activities. While our initial modeling experiments suggested that the Y70A mutation could permit FP to bind in an extended conformation, molecular dynamics simulations particularly of the binding of FP to the active site of YV₇₃I showed that FP could also bind in a kinked conformation in the presence of the Y70A mutation, in a manner that does not fully exploit the available space resulting from the mutations.

Our steady-state measurements showed that YV₁₃₀I, YV₇₃V₁₃₀I and YV₇₃I have K_M^{FP} s that were lower than K_M^{GP} . FP binding might have been enhanced by van der Waals interactions between the long isoprenoid chain and the hydrophobic walls of the active site, and turnover relative to GP limited by unfavorable conformations or slow product release.

We also observed that the kinetic parameters for ATP for the mutants were similar to those of wild-type THA IPK, regardless of the cosubstrate. This is reasonable since the mutations were confined to the hydrocarbon pocket in the IP binding site, and there were no obvious interactions between ATP and the monophosphate substrates aside from their terminal phosphate groups.

Our results complement a previous report by Deltas and Noel in which novel FP kinase activities were achieved by mutagenesis of MJ IPK (7). We have shown that analogous mutations in the THA IPK enzyme would result in GP kinase activities that are stronger than their coexistent FP kinase activities, producing both GPP and FPP. Other groups have reported the existence of isoprenol kinases that are able to catalyze the successive phosphorylation of farnesol and geranylgeraniol to the corresponding diphosphates using ATP, GTP, UTP or CTP as cosubstrates. These enzymes probably salvage isoprenoid alcohols for prenyltransfer reactions (13–17).

Conclusion

Through structure-based redesign of the IP binding site of THA IPK, we constructed four variants with improved catalytic activities for phosphorylation of GP and FP. In each case, the size of the hydrophobic pocket in the IP binding site was expended by replacement of the wild-type amino acid with alanine. These changes resulted in a reduced catalytic efficiency for phosphorylation of IP through a decrease in k_{cat}^{IP} and an increase in K_M^{IP} . While similar decreases were seen for K_M^{GP} for the mutants, their k_{cat}^{GP} s were substantially higher than wild-type IPK. In particular, YV₁₃₀I, where the improvement in k_{cat}^{GP} is >200-fold, is a good catalyst for synthesis of GPP from GP.

Methods

Mutation of THA IPK

Computer modeling was performed using the UCSF CHIMERA software (18) to build GP and FP in the active site of THA IPK with *in silico* mutations. The complex models were then energy-minimized using AMBER 11 (19) with the force field ff03 for protein, and the general AMBER force field (GAFF) for GP and FP. Atomic charges for GP and FP were derived from the AM1–BCC charge scheme using the Antechamber program. Mutagenesis was carried out using the QuikChange Lightning Multi-Site Directed Mutagenesis Kit (Agilent Technologies) and mutagenic primers designed with the Quikchange Primer Design Tool. The plasmid template contained the THA IPK gene in the pET28b vector with an appended N-terminal sequence MGSSHHHHHSSGLVPRGS upstream of the IPK sequence. Mutant constructs were confirmed by sequencing at the University of Utah DNA Sequencing Core Facility.

Detection of GP and FP Kinase Activity

Purified THA IPK mutants were tested for GP, FP and IP kinase activity by incubating 10 μ M of each enzyme with 5 mM of the respective monophosphate in 100 mM HEPES buffer, pH 7.5, containing 10 mM β -mercaptoethanol, 10 mM $MgCl_2$, 1 mg mL^{-1} BSA, 1 μ M [γ -³²P] ATP for 2 h at 37 °C in a total volume of 50 μ L. The mixtures were quenched with 100 μ L of 500 mM EDTA. A 10 μ L to 20 μ L portion of each incubate was spotted on silica plates and developed with $CHCl_3$ /pyridine/formic acid/ H_2O (30:70:16:10 v/v/v/v). The TLC plate was imaged for 24 h using a storage phosphor autoradiography cassette and visualized using a Typhoon 8600 variable mode imager (GE Healthcare).

Kinetic characterization of THA IPK Mutants

The protocol for fluorescent assays was based on the procedure by Pilloff *et al.* with slight modifications (9). The activities of coupling enzymes were determined by measuring the change in absorbance of NADH at 339 nm. Different concentrations of lactate dehydrogenase (LDH) were prepared in 100 mM HEPES buffer, pH 7.5, containing 10 mM MgCl₂, 10 mM β-mercaptoethanol, 1 mg mL⁻¹ BSA, 120 μM pyruvate and 150 μM NADH at 37 °C, and different concentrations of pyruvate kinase (PK) were prepared in 100 mM HEPES buffer, pH 7.5, containing 10 mM MgCl₂, 10 mM β-mercaptoethanol, 1 mg mL⁻¹ BSA, 1 mM PEP, 4 mM ADP, 150 μM NADH and LDH at 37 °C. The rates measured in AU s⁻¹ were converted to specific activity (U mL⁻¹) by using the NADH extinction coefficient $\epsilon = 6.22 \text{ mM}^{-1} \text{ cm}^{-1}$. For kinetic measurements, each solution contained 2.5 units of PK and 3 units of LDH in assay buffer (100 mM HEPES, pH 7.5, containing 10 mM MgCl₂, 10 mM β-mercaptoethanol, 1 mg mL⁻¹ BSA and 250 μM ATP (saturating)), and appropriate amounts of GP, FP or IP. Reactions were initiated by adding mutant enzyme to a final volume of 200 μL. The reaction was monitored at 37 °C for 600 s by observing the change in fluorescence ($\lambda_{\text{ex}} = 340 \text{ nm}$, $\lambda_{\text{em}} = 460 \text{ nm}$) (FluoroMax, Jobin Yvon Horiba). The initial rates were measured from the linear portion of the curve (<15% consumption of the concentration limiting substrate). The kinetic constants were determined by fitting initial rates to equation 1 using non-linear regression in GraFit5 (20)

$$v/[E] = k_{\text{cat}} [S]/[S] + K_M \quad (1)$$

where v is the initial rate, $[E]$ is the total concentration of enzyme in the mixture, $[S]$ is the concentration of the isoprenoid substrate, and K_M is the Michaelis constant. The concentration of ATP was chosen after performing similar experiments using saturating concentrations of the isoprenoid substrates.

UPLC–MS of THA IPK Mutant Products, GPP and FPP

Samples for UPLC–MS of GPP, FPP and IPP produced by THA IPK mutants were obtained by incubating 10 μM of each mutant with 2 mM of GP, FP, or IP (as control) in 100 mM HEPES buffer, pH 7.5, containing 100 mM MgCl₂, 10 mM β-mercaptoethanol, 1 mg mL⁻¹ BSA and 5 mM ATP at 37 °C for 2 h. The mixtures were then centrifuged at 4 °C and 3000 rpm to remove the enzyme using a 10,000 MWCO Centricon (Millipore). The collected fractions were flash frozen and lyophilized overnight, then dissolved in minimum volume of 25 mM NH₄HCO₃. Isoprenoid diphosphate products (GPP, FPP and IPP) were separated from substrates (GP, FP and IP) on a C18 column (for GPP and FPP) and a C4 column (for IPP) on a Waters ACQUITY UPLC H-Class system with TQ (tandem quadrupole) detector. Isocratic elution with 95% 25 mM NH₄HCO₃ and 5% acetonitrile was used to separate GPP and GP, while isocratic elution with 90% 25 mM NH₄HCO₃ and 10% acetonitrile was used to separate FPP and FP, each at a flow rate of 0.6 mL min⁻¹. For IPP and IP, isocratic elution with 100% of 25 mM NH₄HCO₃ was performed. Peaks with masses corresponding to each substrate and product was detected by negative-ion (ES-) MS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

The Archaeal mevalonate (MVA) pathway. Orthologs of the first four enzymes are found in the genomes of Archaea. The two enzymes (grey) required to convert mevalonate phosphate to IPP are generally missing in Archaea. An alternate route in the archaeal MVA pathway (blue) consisting of a putative phosphomevalonate decarboxylase and isopentenyl phosphate kinase has been proposed to complete the pathway.

Figure 2.

Alternate views of the isopentenyl phosphate (IP) binding site showing bulky amino acid residues (yellow sticks) mutated to alanine to accommodate the longer isoprenoid chains of geranyl phosphate (GP) and farnesyl phosphate (FP). Tyr70 and Val73 reside on the α C helix, while Val130 and Ile 140 are found on the β 9 and β 10 strands, respectively. The native substrate, IP is shown as sticks.

Figure 3.

Purification of YV₇₃V₁₃₀I, YV₇₃I, YV₁₃₀I, and V₇₃V₁₃₀I, and autoradiography assays using [γ -³²P] ATP. (a) Denaturing SDS PAGE of the four mutants. The masses of molecular weight markers are indicated. Monomeric THA IPK mutants have masses of ~ 29 kDa. (b) Autoradiogram showing production of GPP (lane 1), FPP (lane 2), and IPP (lane 3) by each of the mutants.

Figure 4. Binding conformations of geranyl phosphate (GP) and farnesyl phosphate (FP) in the active sites of two THA IPK mutants. (a) Mesh representation of the GP binding site of YV₁₃₀I. This mutant exhibited the highest k_{cat}^{GP} among the four THA IPK variants. (b) Mesh representation of the FP binding site of YV₇₃I. The FP molecule is not fully extended in this binding mode, although the Y70A mutation may allow its binding in this conformation.

Table 1

Mutants of *Thermoplasma acidophilum* isopentenyl phosphate kinase (THA IPK) and combinations of mutations in the IP binding site.

Mutant name	Combination of residues mutated to alanine
YV ₁₃₀ I	Tyr70, Val130, Ile140
V ₇₃ V ₁₃₀ I	Val73, Val130, Ile140
YV ₇₃ V ₁₃₀ I	Tyr70, Val73, Val130 Ile140
YV ₇₃ I	Tyr70, Val73, Ile140

Table 2

Kinetic constants of *Thermoplasma acidophilum* isopentenyl phosphate kinase (THA IPK) mutants for the phosphorylation of isopentenyl phosphate (IP), geranyl phosphate (GP), and farnesyl phosphate (FP). Measurements were performed at a saturating concentration of ATP (250 μ M).

	k_{cat} (s^{-1})	K_M (μ M)	k_{cat}/K_M ($M^{-1}s^{-1}$)
Wild-type THA IPK			
GP	0.05	$4.7 (\pm 1.3) \times 10^3$	10.0
FP	not determined	not determined	not determined
IP	8.0	$4.4 (\pm 0.5)$	1.8×10^6
YV₇₃V₁₃₀I			
GP	$1.1 (\pm 0.1)$	$2.4 (\pm 0.3) \times 10^3$	4.7×10^2
FP	$0.6 (\pm 0.1)$	$1.8 (\pm 0.1) \times 10^3$	3.4×10^2
IP	$2.6 (\pm 0.1) \times 10^{-3}$	$5.3 (\pm 0.8) \times 10^3$	0.5
YV₇₃I			
GP	$4.1 (\pm 0.2)$	$3.5 (\pm 0.3) \times 10^3$	1.2×10^3
FP	$1.4 (\pm 0.1)$	$1.6 (\pm 0.3) \times 10^3$	8.8×10^2
IP	$1.0 (\pm 0.6) \times 10^{-2}$	$7.9 (\pm 0.1) \times 10^3$	1.3
YV₁₃₀I			
GP	$10.1 (\pm 0.7)$	$8.0 (\pm 1.1) \times 10^3$	1.3×10^3
FP	$1.4 (\pm 0.1)$	$1.5 (\pm 0.2) \times 10^3$	9.9×10^2
IP	$4.8 (\pm 0.3) \times 10^{-3}$	$5.7 (\pm 0.9) \times 10^3$	0.9
V₇₃V₁₃₀I			
GP	$2.8 (\pm 0.5)$	$9.5 (\pm 2.7) \times 10^3$	3.0×10^2
FP	not determined	not determined	not determined
IP	$1.1 (\pm 0.1) \times 10^{-2}$	$4.2 (\pm 0.7) \times 10^3$	2.6