Characterization and engineering of a plastic-degrading aromatic polyesterase

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Poly(ethylene terephthalate) (PET) is the most abundantly produced synthetic polymers and is accumulating in the environment at a staggering rate as discarded packaging and textiles. The properties that make PET so useful also endow it with an alarming resistance to biodegradation, likely lasting centuries in the environment. Our collective reliance on PET and other plastics means that this buildup will continue unless solutions are found. Recently, a newly discovered bacterium, Ideonella sakaiensis 201-F6, was shown to exhibit the rare ability to grow on PET as a major carbon and energy source. Central to its PET biodegradation capability is a secreted PETase (PET-digesting enzyme). Here, we present a 0.92 Å resolution X-ray crystal structure of PETase, which reveals features common to both cutinases and lipases. PETase retains the ancestral α/β-hydrolase fold but exhibits a more open active-site cleft than homologous cutinases. By narrowing the binding cleft via mutation of two active-site residues to conserved amino acids in cutinases, we surprisingly observe improved PET degradation, suggesting that PETase is not fully optimized for crystalline PET degradation, despite presumably evolving in a PET-rich environment. Additionally, we show that PETase degrades another semiaromatic polyester, polyethylene-2,5-furandicarboxylate (PEF), which is an emerging, bioderived PET replacement with improved barrier properties. In contrast, PETase does not degrade aliphatic polyesters, suggesting that it is generally an aromatic polyesterase. These findings suggest that additional protein engineering to increase PETase performance is realistic and highlight the need for further developments of structure/activity relationships for biodegradation of synthetic polyesters.

In less than a century of manufacturing, plastics have become essential to modern society, driven by their incredible versatility coupled to low production costs. It is, however, now widely recognized that plastics pose a dire global pollution threat, especially in marine ecosystems, because of the ultralong lifetimes of most synthetic plastics in the environment (1–9). In response to the accumulation of plastics in the biosphere, it is becoming increasingly recognized that microbes are adapting and evolving enzymes and catabolic pathways to partially degrade man-made plastics as carbon and energy sources (10–19). These evolutionary footholds offer promising starting points for industrial biotechnology and synthetic biology to help address the looming environmental threat posed by man-made synthetic plastics (19–23).

Poly(ethylene terephthalate) (PET) is the most abundant polyester plastic manufactured in the world. Most applications that employ PET, such as single-use beverage bottles, clothing, packaging, and carpeting, employ crystalline PET, which is recalcitrant to catalytic or biological depolymerization due to the limited accessibility of the ester linkages. In an industrial context, PET can be depolymerized to its constituents via chemistries able to cleave ester bonds (24, 25). However, to date, few chemical recycling solutions have been deployed, given the high processing costs relative to the purchase of inexpensive virgin PET. This, in turn, results in reclaimed PET primarily being mechanically recycled, ultimately resulting in a loss of material properties, and hence intrinsic value. Given the recalcitrance of PET, the fraction of this plastic stream that is landfilled or makes its way to the environment is projected to persist for hundreds of years (1).

In 2016, Yoshida et al. (17) reported a newly discovered bacterium, Ideonella sakaiensis 201-F6, with the unusual ability to use PET as its major carbon and energy source for growth. Especially in the past decade, there have been multiple, foundational studies reporting enzymes that can degrade PET (10, 26–31), but, to our knowledge, previous work had not connected

Significance

Synthetic polymers are ubiquitous in the modern world but pose a global environmental problem. While plastics such as poly(ethylene terephthalate) (PET) are highly versatile, their resistance to natural degradation presents a serious, growing risk to fauna and flora, particularly in marine environments. Here, we have characterized the 3D structure of a newly discovered enzyme that can digest highly crystalline PET, the primary material used in the manufacture of single-use plastic beverage bottles, in some clothing, and in carpets. We engineered this enzyme for improved PET degradation capacity and further demonstrate that it can also degrade an important PET replacement, polyethylene-2,5-furandicarboxylate, providing new opportunities for biobased plastics recycling.

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Conflict of interest statement: H.P.A., M.D.A., B.S.D., N.A.R., C.W.J., J.E.M., and G.T.B. have filed a patent application on the PETase double mutant.

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Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.pdb.org (PDB ID codes 6EOQ, 6EOE, 6EOF, and 6EOH). 1H.P.A., M.D.A., B.S.D., N.A.R., and F.L.K. contributed equally to this work.

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extracellular enzymatic PET degradation to catabolism (11) in a single microbe. As illustrated in Fig. 1, Yoshida et al. (17) demonstrated that an I. sakaiensis enzyme dubbed PETase (PET-digesting enzyme) converts PET to mono(2-hydroxyethyl)terephthalic acid (MHET), with trace amounts of terephthalic acid (TPA) and bis(2-hydroxyethyl)-TPA as secondary products. A second enzyme, MHETase (MHET-digesting enzyme), further converts MHET into the two monomers, TPA and ethylene glycol (EG). Both enzymes are secreted by I. sakaiensis and likely act synergistically to depolymerize PET. Sequence analysis and mutant distal to the catalytic center that we hypothesized would be produced and tested for PET degradation, including a double homologous cutinase active-site cleft (41), PETase variants were created and tested. Based on differences in the PETase and cutinase structures, the PETase has evolved to degrade crystalline PET, it potentially may have promiscuous activity across a range of polyesters. At its widest point, the date crystalline semiaromatic polyester, which is predicted to offset greenhouse gas emissions relative to PET (39), its lifetime in the environment, like that of PET, is likely to be quite long (40). Given that PETase has evolved to degrade crystalline PET, it potentially may have promiscuous activity across a range of polyesters.

In this study, we aimed to gain a deeper understanding of the adaptations that contribute to the substrate specificity of PETase. To this end, we report multiple high-resolution X-ray crystal structures of PETase, which enable comparison with known cutinase structures. Based on differences in the PETase and a homologous cutinase active-site cleft (41), PETase variants were produced and tested for PET degradation, including a double mutant distal to the catalytic center that we hypothesized would alter important substrate-binding interactions. Surprisingly, this double mutant, inspired by cutinase architecture, exhibits improved PET degradation capacity relative to wild-type PETase. We subsequently employed in silico docking and molecular dynamics (MD) simulations to characterize PET binding and dynamics, which provide insights into substrate binding and suggest an explanation for the improved performance of the PETase double mutant. Additionally, incubation of wild-type and mutant PETase with several polyesters was examined using scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and product release. These studies showed that the enzyme can degrade both crystalline PET (17) and PEF, but not aliphatic polyesters, suggesting a broader ability to degrade semiaromatic polyesters. Taken together, the structure/function relationships elucidated here could be used to guide further protein engineering to more effectively depolymerize PET and other synthetic polymers, thus informing a biotechnological strategy to help remediate the environmental scourge of plastic accumulation in nature (19–23).

Results

PETase Exhibits a Canonical α/β-Hydrolase Structure with an Open Active-Site Cleft. The high-resolution X-ray crystal structure of the I. sakaiensis PETase was solved employing a newly developed synchrotron beamline capable of long-wavelength X-ray crystallography (42). Using single-wavelength anomalous dispersion, phases were obtained from the native sulfur atoms present in the protein. The low background from the in vacuo setup and large curved detector resulted in exceptional diffraction data quality extending to a resolution of 0.92 Å, with minimal radiation damage (SI Appendix, Fig. S1 and Table S1).

As predicted from the sequence homology to the lipase and cutinase families, PETase adopts a classical α/β-hydrolase fold, with a core consisting of eight β-strands and six α-helices (Fig. 24). Yoshida et al. (17) noted that PETase has close sequence identity to bacterial cutinases, with Thermobifida fusca cutinase being the closest known structural representative (with 52% sequence identity; Fig. 2B and SI Appendix, Fig. S24), which is an enzyme that also degrades PET (26, 29, 41). Despite a conserved fold, the surface profile is quite different between the two enzymes. PETase has a highly polarized surface charge (Fig. 2C), creating a dipole across the molecule and resulting in an overall isoelectric point (pI) of 9.6. In contrast, T. fusca cutinase, in common with other cutinases, has a number of small patches of both acidic and basic residues distributed over the surface, conferring a more neutral pI of 6.3 (Fig. 2D).

Another striking difference between PETase and the closest cutinase homologs is the broader active-site cleft, which, upon observation, we hypothesized might be necessary to accommodate crystalline semiaromatic polyesters. At its widest point, the cleft in PETase approaches threefold the width of the corresponding structure in the T. fusca cutinase. The expansion is achieved with minimal rearrangement of the adjacent loops and secondary structure (Fig. 2 E and F). A single amino acid substitution from phenylalanine to serine in the lining of the active-site cavity appears sufficient to cause this change, with the remaining cleft formed between Trp159 and Trp185 (Fig. 2G). This relative broadening of the active-site cleft is also observed in comparisons with other known cutinase structures (SI Appendix, Fig. S3 A–D).

In terms of the active site, the well-studied catalytic triad is conserved across the lipases and cutinase families (43). In PETase, the catalytic triad comprises Ser160, Asp206, and His237, suggesting a charge-relay system similar to that found in other α/β-fold hydrolases (44). The specific location and geometry between the active site found in cutinases is also conserved in PETase (Fig. 2 G and H and SI Appendix, Fig. S4). In common with most lipases, the catalytic residues reside on loops, with the nucleophilic serine occupying a highly conserved position known

![PETase catalyzes the depolymerization of PET to bis(2-hydroxyethyl)-TPA (BHET), MHET, and TPA. MHETase converts MHET to TPA and EG.](image-url)
as the nucleophile elbow (45). The nucleophilic serine sits in the consensus sequence (Gly-X1-Ser-X2-Gly), and while this “lipase box” is common to most lipases (SI Appendix, Fig. S4A) and cutinases (SI Appendix, Fig. S4B), the X1 position, usually occupied by a histidine or phenylalanine in cutinases and lipases, contains a tryptophan residue, Trp159, in PETase (46) (Fig. 2G). This residue has the effect of extending the hydrophobic surface adjacent to the active site (SI Appendix, Fig. S3 E and F). In common with the Fusarium solani cutinase, PETase has two disulfide bonds, one adjacent to the active site and one near the C terminus of the protein. MD simulations have predicted that the active-site disulfide in F. solani cutinase is important for active-site stability, and it may play a similar role in PETase (47).

To explore the potential effects of crystallization conditions and packing effects, three additional crystallography datasets ranging in resolution from 1.58 to 1.80 Å provided a total of seven independent PETase chains (SI Appendix, Table S1). All domains adopt the same fold (relative rmsd values are ~0.28 Å), and all of the residues of the catalytic triad exhibit the same conformation (SI Appendix, Fig. S5 A and B), including Trp159. In one crystal form, however, Trp185 was present in the active-site cleft of PETase active site is shown with the width between Thr61 and Phe209 in equivalent positions. (G) Close-up view of the PETase active site with the catalytic triad residues His237, Ser160, and Asp206 colored blue. Residues Trp159 and Trp185 are colored pink. The residues in PETase colored pink correspond to the site-directed mutagenesis targets S238F, W159H, and W185A.

Converting PETase to a Cutinase-Like Active-Site Cleft Enables Improved Crystalline PET Degradation. From the PETase structure, we originally hypothesized that changes in the active site relative to the T. fusca cutinase resulted from the evolution of I. sakaiensis in a PET-containing environment, thus enabling more efficient PET depolymerization. To test this hypothesis, we mutated the PETase-active site to make it more cutinase-like. Specifically, a double mutant was produced, S238F/W159H, which, based on homology modeling, was predicted to narrow the PETase active site, similar to the T. fusca cutinase. Additionally, we produced the W185A mutant to examine the role of this highly conserved dynamic residue.

In the original report describing the discovery of PETase, Yoshida et al. (17) examined PETase digestions of amorphous PET films with a crystallinity of 1.9%, which is lower than that of most PET samples that would be encountered either in the environment or in an industrial recycling context (48). To examine the performance in the wild-type PETase relative to the two mutants, we examined PET digestion with coupons of higher crystallinity. Specifically, PET coupons with an initial crystallinity of 14.8 ± 0.2% (for reference, a commercial soft drink bottle examined via the same methods exhibits a crystallinity of 15.7% as measured by DSC) were synthesized and characterized by NMR spectroscopy to confirm their structure and by DSC to determine their crystallinity (SI Appendix, Fig. S6A). Digestions were conducted at pH 7.2 and monitored with DSC, NMR spectroscopy, and SEM, and reaction products were quantified by HPLC and NMR spectroscopy. Fig. 3 A–D shows the results of PET degradation, including a buffer-only control, the wild-type PETase, and the double mutant. It is clear that PETase induces surface erosion and pitting of a PET film with a crystallinity of 13.3 ± 0.2%, resulting in a 10.1% relative crystallinity reduction (absolute reduction of 1.5%; SI Appendix, Table S2). Surprisingly, the PETase double mutant outperforms the wild-type PETase by both crystallinity reduction and product release. The absolute crystallinity loss is 4.13% higher, and the corresponding SEM images appear to show that slightly more surface ablation occurs (Fig. 3C). After incubation, the digested PET samples for both the wild-type PETase and the double mutant exhibit a lower melting temperature over a wider temperature range.
range (SI Appendix, Fig. S6F), indicating that the crystalline domain regions are reduced in size.

Understanding how PET binds in the PETase catalytic site is key to understanding the improved performance of the PETase double mutant. We attempted multiple trials to obtain a ligand-bound structure of PETase, to no avail. While this paper was in revision, Han et al. (32) published a study with an R132G/S160A mutant (R132 and S160 in the numbering here) that was able to accommodate monomeric ligands, alongside the publication of another PETase structure soon after (33). Here, we sought to predict PET-PETase binding modes by conducting induced fit docking (IFD) (SI Appendix, Fig. S7A). Multiple PET orientations were predicted by IFD in and around the active site of both the wild-type and double-mutant enzymes. The orientation shown in Fig. 3E and SI Appendix, Fig. S7A is one of several used to illustrate a productive PET-binding event in the wild-type enzyme: A PET carboxyl carbon is at a chemically relevant distance (5.1 Å) for nucleophilic attack from the Ser160 hydroxyl group (49, 50), His237 is at an ideal distance (3.9 Å) to activate Ser160, and Asp206 provides hydrogen bonding support to His237 (2.8 Å). This binding mode is predicted to have binding affinity (estimated by the docking score with descriptors in SI Appendix, Table S3) of −8.23 kcal/mol. Thus, our IFD-predicted binding modes are consistent with a productive Michaelis complex for PET chain cleavage. Additionally, with this low-energy, catalytically competent pose generated from flexible docking (i.e., IFD), we observe a marked difference in the position of Trp185 compared with the crystal structure (SI Appendix, Fig. S7I). The N-Cα-Cβ-C dihedral in the crystal PETase structure is −177.5°, whereas our predicted catalytically competent binding mode of PET indicates W185 rotates to accommodate aromatic interactions with PET, and thus adopts a dihedral angle of 98.4°. This dihedral rotation was observed to various extents in all docking results and in apo MD simulations (SI Appendix, Fig. S5), and thus illustrates the necessity for flexible protein treatment during ligand binding mode prediction, especially if binding and/or catalytic hypotheses are to be posited.

IFD results also suggest potential reasons for the improved performance of the PETase double mutant over wild-type PETase, as the substrate may interact with Phe238 through several aromatic interactions of interest between PET and Phe238 are at optimal distance (each at 5.4 Å).
interactions, as shown in Fig. 3F. In this predicted pose (docking score of $-11.25$ kcal/mol, with descriptors in SI Appendix, Table S3), a PET carbonyl is at an appropriate attack distance from Ser160 (3.1 Å). Ser160 is in the range for deprotonation by His237 (2.9 Å; SI Appendix, Fig. S7C), and Asp206 is ready to accept a proton in the shuttle (2.9 Å). PET aromatic rings are within ideal π-stacking distances (51) to binding site residues (W185 and Y87), and, in particular, two aromatic interactions are formed to Phe238 (point-to-face interaction at 5.4 Å and parallel displaced interaction at 5.4 Å). The marked difference in predicted binding affinities between wild-type and double-mutant enzymes for PET is consistent with the increased activity of the PETase double mutant on PET, as observed experimentally, and we can identify aromatic interactions supported by the S238F mutation as being integral to this enhancement. All aromatic ring-ring distances for described binding modes are illustrated in SI Appendix, Fig. S7 A and C.

In contrast to the double mutant, the W185A mutant exhibits highly impaired performance relative to the wild-type PETase, as described in SI Appendix, Fig. S5 G–J and Table S2. These data confirm a critical role for this residue. From the IFD, Trp185 is predicted to play an important role by contributing π-stacking interactions to PET aromatic groups. Additionally, in all productive binding modes (i.e., when the carbonyl is oriented to be in the oxyanion hole and the carbonyl carbon is at a catalytic distance from Ser160), Trp185 is predicted to reorient relative to the crystal structure, suggesting its movement opens the active-site cleft, allowing PET binding (SI Appendix, Fig. S7I).

**PETase Depolymerizes PEF, but Not Aliphatic Polyesters.** We were also interested in understanding the activity of wild-type PETase and the PETase double mutant on other polymeric substrates, including aliphatic and other semiaromatic polyesters. To that end, we synthesized, characterized (SI Appendix, Fig. S6 C and D), and conducted similar incubations with the aliphatic polyesters PBS and PLA. None of these samples showed visual differences between the control images and the PETase-treated samples.
samples (SI Appendix, Fig. S8), suggesting that PETase and the double mutant are not active on aliphatic polyesters.

PET is another semiaromatic polyester marketed as a bio-based PET replacement (38, 39). Given the structural similarity of PET and PEF, and recent studies on PEF degradation by cutinases (52), we hypothesized that PETase may also depolymerize this substrate. Accordingly, we synthesized PEF coupons, and Fig. 4 A–D shows the results of PEF incubations with the wild-type PETase enzyme and the PETase double mutant, alongside a buffer-only control. Visually, the surface morphology of PETase-treated PEF is even more modified than PET, with SEM revealing the formation of large pits, suggesting that PETase is potentially much more active on this substrate than PET. The observation of enhanced PET degradation by microscopy is corroborated by the DSC data for PEF, which show a reduction in relative crystallinity of 15.7% (absolute of 2.4%) compared with a relative reduction of 10.1% for PET (SI Appendix, Fig. S6E and Table S2).

To predict how a PEF oligomer interacts with the wild-type and double-mutant PETase-active sites, IFD was again performed. The expected PETase activity was again captured from a structural standpoint, with the PET ester oriented within nucleophilic attack distance of Ser160 (Fig. 4 E and F and SI Appendix, Fig. S7B). As with PET IFD results, we were able to identify interactions to support increased activity of the PETase double-mutant enzyme. In the PEF wild-type binding mode (docking score of ~9.07 kcal/mol), two aromatic interactions are formed to Trp185 and Trp159 (SI Appendix, Fig. S7B). However, in the PEF double-mutant binding mode (docking score of ~10.07 kcal/mol), three aromatic interactions were observed: parallel displaced to Trp185 (5.7 Å), point to His237 (5.1 Å), and parallel displaced to Phe238 (5.2 Å). Additionally, Tyr87 is within range for a potential aromatic interaction at 6.2 Å. One interesting interaction was observed in the PEF double-mutant binding mode: His237 flipped “up,” out of the catalytic triad, to play an aromatic stabilization role (replacing the wild-type Trp159 stabilization), and, instead, His159 supported Ser160 via hydrogen bonding at 3.2 Å. This interaction between Ser160 and His159 is also observed in apo MD simulations of the double-mutant structure. It could thus be postulated that His159 serves as an additional means for shuttling protons in the PETase double mutant. Binding of PET and PEF to the double-mutant PETase (SI Appendix, Table S3), and, structurally, we can relate this to aromatic interactions supported by PEF and a potential alternative pathway for proton shuttling during catalysis.

Discussion

The high-resolution structure described in the present study reveals the binding site architecture of the I. sakaiensis 201-F6 PETase, while the IFD results provide a mechanistic basis for both the wild type and PETase double mutant toward the semiaromatic polyesters PEF and PET. Changes around the active site result in a widening of the cleft compared with structural representatives of three thermophilic cutinases (SI Appendix, Fig. S3), without other major changes in the underlying secondary or tertiary structure. Furthermore, we demonstrated that PETase is active on PET of ~15% crystallinity; while this observation is encouraging, it is envisaged that its performance would need to be enhanced substantially, perhaps via further active-site cleft engineering similar to ongoing work on thermophilic cutinases and lipases (26, 30, 53, 54). Enzyme scaffolds capable of PET breakdown above the glass transition temperature (≥70 °C for PET) (20) will also be pursued in future studies. Coupling with other processes such as milling or grinding, which can increase the available surface area of the plastic, also merits investigation toward enzymatic solutions for PET and PEF recycling. Furthermore, in light of recent studies that demonstrate the impressive synergistic effect of combining multiple PET-active lipases (26, 30, 53, 54), we expect that incorporation of I. sakaiensis MHETase will further increase the performance (55), and this will be pursued in future work. The highly basic surface charge of PETase requires further investigation since it is not observed in other close structural homologs, but it is noteworthy that the MHETase partner is predicted to be a fairly acidic protein, with a pI in the region of 5.2.

Both the IFD results and MD simulations independently indicate the PETase binding site is characterized by highly flexible, large aromatic side chains, such as Trp185, Tyr87, and Trp159, and Phe238 in the PETase double mutant. Binding of PET and PEF induces conformational changes in these residues relative to the crystal structure; thus, modeling protein flexibility in response to PET/PEF is critical to predict catalytically relevant binding modes. Additionally, results of these flexible docking studies agree with experimentally observed trends in performance in the wild type relative to the double mutant, and provide structural insight to explain this enhancement.

PETase activity on both PET and PEF, but not on aliphatic polyesters such as PBS and PLA, provides the basis for characterizing this enzyme more broadly as an aromatic polyesterase rather than solely as a PETase. It is likely that the enhanced gas barrier properties of PEF will lead to its adoption for beer bottles, and that this recalcitrant material will thus ultimately find its way to the environment. It is therefore encouraging that PETase is also natively capable of PEF degradation. It is also noteworthy that in this study, PETase was freeze-dried and shipped between continents, and that it retained similar performance profiles after freeze-drying, which is a positive feature for its potential use in applications that require enzyme production and use be distinct, as it would potentially be the case for most biobased recycling options.

The problem of plastics depolymerization by enzymes closely mirrors that of enzymes that depolymerize polysaccharides, such as cellulose and chitin (56, 57). Indeed, strategies that have been used to understand and improve glycoside hydrolases, including the development of quantitative assays for measuring enzyme (or enzyme cocktail) performance on solid substrates, likely can serve as inspiration for more quantitative metrics for comparing plastic-degrading enzymes and enzyme mixtures, which will be reported in future studies. Moreover, the method of PETase action is of keen interest for further protein and enzyme mixture engineering studies. The direct catalytic mechanism could be studied with mixed quantum mechanical/molecular mechanics MD-based approaches similar to previous work on carbohydrate-active enzymes (58). Beyond the active site, the enzyme may interact with and cleave the substrate in an endofashion by cleaving PET (or PEF) chains internal to a polymer or in an exofashion by only cleaving PET from the chain ends. Methods employed in the cellulase and chitinase research community, such as substrate labeling with easily detected reporter molecules or examination of product ratios, could potentially shed light on this question, and will be pursued in future efforts (59). Lastly, at low substrate loadings, many polysaccharide-active enzymes rely on multimodular architectures, with a carbohydrate-binding module attached to the catalytic domain (57). For polyesterase enzymes, hydrophobins, carbohydrate-binding modules, and polyhydroxyalkanoate-binding modules have been used to increase the catalytic efficiency of cutinases for PET degradation (60, 61). Certainly, further opportunities exist for engineering or evolving for higher binding affinity of accessory modules to increase the overall surface concentration of catalytic domains on the PET surface.

Given the fact that PET was only patented roughly 80 y ago and put into widespread use in the 1970s, it is likely that the enzyme system for PET degradation and catabolism in I. sakaiensis appeared only recently, demonstrating the remarkable evolution of an ancient enzyme system to a new and modern role.
speed at which microbes can evolve to exploit new substrates: in this case, waste from an industrial PET recycling facility. Moreover, given the results obtained for the PETase double mutant, it is likely that significant potential remains for improving its activity further. This enzyme thus provides an exciting platform for additional protein engineering and evolution to increase the efficiency and substrate range of this polyesterase, as well as to provide clues of how to further engineer thermophilic cutinases to better incorporate aromatic polystyres, toward to the persistent challenge of highly crystalline polymer degradation.

Conclusions

The discovery of a bacterium that uses PET as a major carbon and energy source has raised significant interest in how such an enzymatic mechanism functions with such a highly resistant polymeric substrate that appears to survive for centuries in the environment. This work shows that a collection of subtle variations on the surface of a lipase/cutinase-like fold has the ability to endow PETase with a platform for aromatic polyester depolymerization. These findings open up the possibility to further utilize and combine the extensive platform of cutinase and lipase research over the past decades with directed protein engineering and evolution to adapt this scaffold further and tackle environmentally relevant polymer bioaccumulation and biobased industrial polyester recycling.

Methods

Cloning and Protein Production. Codon optimized Escherichia coli expression clones were constructed for PETase as described in SI Appendix, Fig. S28.

Crystallization and Structure Determination. PETase was crystallized in five conditions, and long-wavelength sulfur-single-wavelength anomalous diffraction and high-resolution X-ray data collection was performed in vacuo at beamline ID13. Standard X-ray data collection was performed at beamlines I03 and I04 at the Diamond Light Source. Detailed methods and statistics are provided in SI Appendix, Table S1.

Substrate Docking. The PETase crystal structure, PETase double mutant, and PET and PEF tetramers were modeled using tools from Schrödinger. PETase double mutant, and PET tetramer were modeled using tools in Schrödinger. PET and PEF tetramers were modeled using tools from Schrödinger. Substrate Docking.

PETase Digestion of Polymer Films. Coupons sized ~6 mm in diameter of each polymer film were placed in a 1.5-M L Eppendorf tube with 500 μL of 50 mM PETase in 50 mM phosphate buffer at pH 7.2. The digestions were carried out at 30 °C. Analysis of the films and supernatant was done after 96 h of digestion.

SEM. Polymer coupons sized ~6 mm in diameter were examined by SEM, both before and after PETase treatment for 96 h. PETase-treated samples were rinsed with 1% SDS, followed by dH2O and then ethanol. Samples were sputter-coated with 8 nm of iridium. Coated samples were mounted on aluminum stubs using carbon tape, and conductive silver paint was applied to the sides of the samples to reduce charging. SEM imaging was performed using an FEI Quanta 400 FEG instrument under low vacuum (0.45 torr) operating with a gaseous solid-state detector. Imaging was performed with a beam-accelerating voltage of 15 keV.

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