A Family 2 Pectate Lyase Displays a Rare Fold and Transition Metal-assisted β-Elimination*

Received for publication, July 5, 2007, and in revised form, September 10, 2007
Published, JBC Papers in Press, September 19, 2007, DOI 10.1074/jbc.M705511200

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The family 2 pectate lyase from Yersinia enterocolitica (YePL2A), solved to 1.5 Å, reveals it to be the first prokaryotic protein reported to display the rare (α/α)_7 barrel fold. In addition to its apo form, we have also determined the structure of a metal-bound form of YePL2A (to 2.0 Å) and a trigalacturonic acid-bound substrate complex (to 2.1 Å). Although its fold is rare, the catalytic center of YePL2A can be superimposed with structurally unrelated families, underlining the conserved catalytic amino acid architecture of the β-elimination mechanism. In addition to its overall structure, YePL2A also has two other unique features: 1) it utilizes a metal atom other than calcium for catalysis, and 2) its Brønstead base is in an alternate conformation and directly interacts with the uronate group of the substrate.

Recently, a conserved pectin utilization pathway was discovered in a variety of human pathogens from Enterobacteracae (1). This pathway is an abridged form of the efficient and highly decorated extracellular pectin degradation systems found in soft rot-causing bacteria from the genus Erwinia (2). Pectin is a heterogeneous polysaccharide primarily composed of an α-D-1,4-linked polygalacturonic acid backbone. The main function of this carbohydrate is to stabilize structural polysaccharides such as cellulose and xylan, providing rigidity to the cell wall. Degradation of this structural “glue” by the enzymes of plant pathogens therefore results in the characteristic cell necrosis and tissue maceration during soft rot infections.

The majority of structural biology within this field has focused on the primary secreted agents of pathogenesis, which include pectate lyases (3, 4), polygalacturonases (5, 6), and pectin methylesterases (7, 8). Although these enzymes utilize distinct catalytic mechanisms, they commonly display a right-handed parallel β-helix topology (9). There is a notable exception, however, because family 10 pectate lyases adopt an (α/α)_7 toroid conformation (10, 11). Interestingly, superimposition of bound oligogalacturonide substrates within the active sites of these two different pectate lyase folds showed that despite the disparity in overall structure, there is remarkable similarity in the architecture of the catalytic residues (10). This suggested that the process of galacto-configured uronic acid β-elimination by distinct enzymes demands a conserved framework within the active site.

Following polygalacturonic acid depolymerization, oligogalacturonides are shuttled into the periplasm through a specific porin, KdgM (12, 13). Within the periplasmic compartment there are commonly four conserved proteins present to further process oligo- and polygalacturonic acid substrates for intracellular transport (1, 14, 15) that we have structurally and functionally characterized. These proteins include a family 32 carbohydrate-binding module (CBM32) (16); a family 2 polysaccharide lyase (PL2A) and a family 28 glycoside hydrolase (GH28) (17), both of which are pectinases; and TogB, the specificity determinant of the TogMNAB ABC class inner membrane oligogalacturonide transporter (18).

Here we report the three-dimensional structure of the PL2A from Yersinia enterocolitica (YePL2A), which is the first (α/α)_7 barrel fold ever found in a prokaryotic protein. In addition we have determined the structure of this protein in complex with a metal cofactor and a trigalacturonic acid (trigalUA) substrate and compared its activity with its closely related cytoplasmic family 2 homolog, YePL2B.

EXPERIMENTAL PROCEDURES

Cloning, Gene Expression, and Protein Production and Purification—The open reading frames encoding YePL2A and YePL2B were identified in the genome sequence of Y. enterocolitica 8081 (www.sanger.ac.uk/Projects/Y_enterocolitica/) using the full-length Yersinia pestis CO92 family 2 pectate lyase gene sequences available within the CAZy data base (www.cazy.org) (19) as search templates. PCR primers were designed based on the Y. enterocolitica 8081 sequences, and in the case of YePL2A, the N-terminal sequence tag that was identified using SignalP (20) was omitted. The gene fragment encoding YePL2A was amplified with CATATGGC- TAGCGGGATACCAGCCGCTG (YePL2A forward primer) and GAATTCTCGAGTTATTTACGGCCGT-TCGGTTG (YePL2A reverse primer). Likewise, the gene

* This work was supported by a grant from the Canadian Institutes of Health Research. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The atomic coordinates and structure factors (code 2V8I, 2V8J, and 2V8K) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

The on-line version of this article (available at http://www.jbc.org) contains supplemental Fig. S1.

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2 The abbreviations used are: YePL2A, Y. enterocolitica pectate lyase 2A; YePL2B, Y. enterocolitica pectate lyase 2B; trigalUA, trigalacturonic acid; CAPS, 3-(cyclohexylamino)propanesulfonic acid; CAPSO, 3-(cyclohexylamino)propanesulfonic acid sodium salt.
The product samples were subsequently thawed and loaded into 3.0. The reactions were stopped with EDTA and flash frozen. A solution of polygalacturonic acid dissolved in CAPSO, pH 9.0, was used as a template. The amplified DNA fragments were cloned via the engineered 5′ NheI and 3′ Xhol restriction sites into identically digested pET28a to yield the plasmids pETPL2A and pETPL2B, which encode the YePL2A and YePL2B proteins, respectively, fused to N-terminal 6-histidine tag by thrombin protease cleavage sites. All PCR, cloning, and protein production and purification protocols for YePL2A and YePL2B have been described previously in detail for other proteins (21).

**Enzyme Assays**—Optimal pHs for YePL2A and YePL2B were determined in reactions adapted from Shevchik et al. (22) by incubating 0.05% polygalacturonic acid and 1 mM trigalUA, respectively, with 10 nm of enzyme. The reactions were monitored at 232 nm for the formation of unsaturated bonds in a broad range of pH conditions (6–11) to determine the region of optimum activity. Subsequently, a more accurate analysis was determined by performing a stepwise scan with intervals increasing by 0.2 pH units using buffers: Tris-HCl (8.0–8.8), CAPSO (9.0–9.8), and CAPS (10.0–11.0). The optimal pH conditions of 9.6 for YePL2A and 8.6 for YePL2B were then utilized for all subsequent enzyme activity experiments.

Metal depletion assays were performed at optimal substrate concentrations (1 mM trigalUA) and run over a time course of 3 min. EDTA concentrations of 10 mM were used to ablate enzyme activity. Metal supplementation was analyzed at 40 mM final concentration.

Substrate specificities were determined by incubating 10 nm enzyme with increasing amounts of substrate dissolved in deionized sterilized water. All of the runs lasted 3 min and were performed in triplicate for statistical significance. Product quantification was performed using an extinction coefficient of 5,200 M−1 cm−1 (22).

Product profiling was performed by analyzing products generated from YePL2A and YePL2B (1 μM) digestion of a 1% solution of polygalacturonic acid dissolved in CAPSO, pH 9.0. The reactions were stopped with EDTA and flash frozen. The product samples were subsequently thawed and loaded directly onto a DIONEX™ HPAEC instrument fitted with a CarboPac PA-20 column (060142; DIONEX, Sunnydale, CA) and pulsed amperometric detection. The retention times were determined in a filtered 0–1% NaOAc gradient, in 0.1 M NaOH using saturated mono-, di-, and trigalUA as standards (not shown).

**Crystallization, Data Collection, and Phasing of YePL2A**—All of the crystallizations were done by the hanging drop vapor diffusion method at 18 °C. YePL2A at 15 mg/ml was crystallized in 30% polyethylene glycol 1500 and 0.1 mM sodium citrate, pH 5.0. X-ray diffraction data of native YePL2A were collected at 113 K by freezing the crystal directly in the cryo-stream after a brief soak in mother liquor supplemented with 10% ethylene glycol. Iodide derivatization of YePL2A was achieved by growing crystals in the above condition with 10% ethylene glycol and 0.1 M NaI. These crystals were then soaked for 1 h in mother liquor containing 10% ethylene glycol and 1.0 M NaI. The soaked crystals were mounted directly in the cryo-stream.

Diffraction data were collected with a Rigaku R-AXIS IV++ area detector coupled to a MM-002 x-ray generator with Osmic “blue” optics and an Oxford Cryostream 700 cryo-cooler. The data were processed using Crystal Clear/d*trek (23). Using both anomalous and isomorphous signal ShelXD (24) found seven iodide sites that were used for phasing by SIRAS within SHARP. Examination of the residual maps after heavy atom refinement and phasing yielded the positions of 11 additional iodide sites. Refinement of the 18 partially occupied iodide sites using data to 1.9 Å yielded isomorphous phasing powers of 0.9 and 1.0 for centric and acentric reflections, respectively, and an anomalous phasing power of 1.2. Figure-of-merits were 0.3 and 0.4 for centric and acentric reflections, respectively. Solvent flattening with a solvent content of 55% resulted in a figure-of-merit of 0.8. ARP/wARP (25) was able to correctly build a virtually complete model with docked side chains. The model was completed manually and refined against the 1.5 Å data with REFMAC (26). Water molecules were added using the ARP/wARP (25) option within REFMAC and inspected visually prior to deposition. In all of the data sets, 5% of the observations were flagged as “free” (27) and used to monitor refinement procedures. Manual model building was performed using COOT (28) and refinements using REFMAC.

The substrate complex was generated by soaking crystals grown in the same conditions as the native crystals (i.e. without NaI) with 1 M trigalUA for 2 h. The structure was solved by refining the apo form model against the complex data set. The trigalacturonic ligand was manually built using COOT. Water molecules were added as above.

The metal-complexed form of YePL2A was crystallized in 25% polyethylene glycol 1500 without buffer. This crystal was mounted directly in the cryo-stream without cryo-protection. This structure was subsequently solved by molecular replacement using MolRep (29) and the apo form coordinates as a search model. The conformation of the protein was sufficiently different to require rebuilding of large parts of the model. This was achieved using ARP/wARP, which was able to correctly build a virtually complete model with docked side chains. Manual completion of the model and addition of waters was performed as above.

All of the data collection, processing, and final model statistics are given in Table 1. The structure factors and atomic coordinates for the NaI derivative, the trigalUA complex, and the metal-bound form have been deposited with the Protein Data Bank under codes 2V8I, 2V8K, and 2V8J, respectively.

**RESULTS AND DISCUSSION**

Enzyme Activity of Family 2 Pectate Lyases—YePL2A displayed a pH optimum of 9.6 on polygalacturonic acid, whereas YePL2B was most efficient on trigalUA at pH 8.6 (Fig. 1, top left panel). This observation is suggestive of adaptation to their different cellular localizations. To characterize their metal dependencies, purified enzyme was utilized in a control reaction, and...
then activity was ablated with EDTA. The inhibited reactions were next supplemented with various transition metals, and the activity was monitored at 232 nm to detect the generation of unsaturated products. Although YePL2A activity was ablated upon the addition of EDTA (not shown), supplementation with various metals resulted in precipitation and prohibited the accurate measurement of activity. In contrast, the closely related YePL2B performed well in this assay and clearly displayed the highest recovery of activity in the presence of Mn$^{2+}$ (Fig. 1, top right panel).

To analyze the product profiles of YePL2A and YePL2B, polygalacturonic acid was digested with each enzyme, and the products were analyzed by HPAEC. YePL2A produced both saturated and unsaturated oligogalacturonides with varying degrees of polymerization from 2–5 subunits in length (Fig. 1, middle left panel). Y. enterocolitica was previously determined to have an endo-acting pectate lyase; however, within this report there was no reported DNA or protein sequence (30). The results presented here suggest that YePL2A is indeed the enzyme in this original report. In contrast, YePL2B almost exclusively produced unsaturated digalacturonic acid, indicating its exo-acting mode of activity (Fig. 1, middle right panel). This was consistent with its similarity to the PelW enzyme from Erwinia chrysanthemi, which was previously identified as an endo-acting pectate lyase (31).

A more thorough kinetic analysis of the activity of these two enzymes on trigalUA and polygalacturonic acid revealed that these enzymes are optimally active on carbohydrates with different degrees of polymerization. YePL2A is preferentially active upon polygalacturonic acid and YePL2B on trigalUA (Fig. 1, bottom left and right panels). Interestingly, both YePL2A and YePL2B appear to display substrate inhibition profiles (Fig. 1, bottom left and right panels), which is not uncommon for pectate lyases (32, 33).

The predicted localization of YePL2A and YePL2B within the pectin utilization pathway is consistent with their observed activities. Polygalacturonic acid is predicted to be bound by the periplasmic protein YeCBM32, which likely functions to retain the polysaccharide within the periplasm (16). Polygalacturonic acid is then depolymerized by YePL2A (YePL2A is active on polygalacturonic acid that is complexed by YeCBM32; data not shown) and YeGH28 to produce small oligogalacturonides 2–3 residues in length (17). These products are the preferential ligands for TogB, the specificity determinant of the intracellular transporter TogMNAB (18). Within the cell, YePL2B would therefore only be presented with smaller substrates and likely operates in a “clean-up” role to convert residual trigalUA into 4,5-unsaturated digalacturonic acid. This end product is the substrate for the downstream oligogalacturonate lyase, Ogl (22), which catalyzes the β-elimination of saturated and unsaturated digalacturonides and initiates the final stages of intracellular pectin catabolism. The preferential activity of YePL2B on trigalUA over PGA, therefore, is in agreement with its inherent exo-activity. The higher activity on the smaller substrate observed is due to the higher molar ratio of terminal ends.

The Overall Structure of YePL2A—The structure of YePL2A was solved by Iodine-SIRAS to a resolution of 1.5 Å and refined with $R$ and $R_{free}$ values of 0.22 and 0.24, respectively (see Table 1 for all data and structure statistics). The core of this enzyme consists of a rare ($\alpha/\alpha$) barrel fold (Fig. 2A), which documents the $\beta$-elimination of saturated and unsaturated digalacturonides and initiates the final stages of intracellular pectin catabolism. The predicted localization of YePL2A and YePL2B within the periplasm (16).

| Data collection | Native | Nal | TrigalUA | No buffer |
|-----------------|--------|-----|----------|-----------|
| Space group     | P2$_1$,2,2$_1$ | P2$_1$,2,2$_1$ | P2$_1$,2,2$_1$ | P2$_1$,2,2$_1$ |
| Cell dimensions | 58.3, 95.9, 126.5 | 58.5, 95.6, 126.6 | 58.4, 95.1, 127.4 | 68.0, 77.7, 103.5 |
| a, b, and c (Å) | 20.00–1.90 (1.97–1.90) | 19.85–1.50 (1.55–1.50) | 19.87–2.10 (2.17–2.10) | 19.91–2.00 (2.07–2.00) |
| $R_{merge}$     | 0.062 (0.364) | 0.053 (0.359) | 0.089 (0.414) | 0.091 (0.371) |
| I/ol            | 10.7 (2.7) | 11.5 (2.9) | 8.6 (2.4) | 8.5 (2.9) |
| Completeness (%) | 99.1 (99.3) | 97.4 (92.2) | 94.6 (98.8) | 93.2 (97.8) |
| Redundancy      | 3.6 (2.8) | 4.4 (4.2) | 4.4 (4.0) | 4.4 (4.0) |

| Refinement | | | | |
| Resolution (Å) | 1.50 | 2.10 | 2.00 |
| No. reflections | 105592 | 174522 | 153616 |
| $R_{merge}/R_{free}$ | 0.22/0.24 | 0.19/0.26 | 0.21/0.25 |
| No. atoms | Protein | 4362 | 4332 | 4262 |
| Ligand/ion | 50 (I) | 37 (ADA) | 1 (Mn) |
| Water | 760 | 589 | 346 |
| B-factors | Protein | 18.5 | 28.9 | 24.6 |
| Ligand/ion | 17.6 | 50.8 | 18.5 |
| Water | 34.2 | 39.9 | 24.9 |
| Root mean square deviations | Bond lengths (Å) | 0.009 | 0.011 | 0.012 |
| Bond angles (°) | 1.144 | 1.284 | 1.25 |

| Ramachandran values | | | |
| Most favored | 91.9% | 91.9% | 91.6% |
| Additionally allowed | 7.9% | 7.9% | 8.2% |
| Generously allowed | 0.2% | 0.2% | 0.2% |
| Disallowed | 0.0% | 0.0% | 0.0% |

| Structure of a Family 2 Pectate Lyase |
|--------------------------------------|
| TABLE 1 Crystallization and refinement statistics |
|--------------------------------------|

The Overall Structure of YePL2A—The structure of YePL2A was solved by Iodine-SIRAS to a resolution of 1.5 Å and refined with $R$ and $R_{free}$ values of 0.22 and 0.24, respectively (see Table 1 for all data and structure statistics). The core of this enzyme consists of a rare ($\alpha/\alpha$) barrel fold (Fig. 2A), which documents the $\beta$-elimination of saturated and unsaturated digalacturonides and initiates the final stages of intracellular pectin catabolism. The predicted localization of YePL2A and YePL2B within the periplasm (16).
constitutes the core of the barrel. This α-barrel appears to function as a structural platform upon which are “grafted” two large arms, which harness the catalytic machinery. These arms consist predominantly of β-strands and define the walls of the active site channel, giving the enzyme an overall “vice-like” shape (Fig. 2B). The distance running between the two arms is ~15 Å across and ~50 Å long, easily large enough to accommodate an oligogalacturonide substrate. The channel is open at both ends, consistent with its observed endo-mode of action (see below).

The (α/α)7 barrel fold has only been previously reported for the family 47 glycoside hydrolases (GH47) (34–37) (Fig. 2, C and D). These enzymes are exclusive eukaryotic mannosidases involved in N-glycan maturation and quality control during glycoprotein synthesis (38). In addition to the difference in the biological function of these enzymes, they operate by completely different mechanisms and are active upon stereochemically distinct substrates. Pectate lyases cleave glycosidic linkages by abstraction of the aglycon H5 and generate a planar product with an unsaturated bond between C-4 and C-5. GH47s cleave glycosidic linkages by the addition of water, resulting in stereochemical inversion around the anomeric carbon. The GH47 from Saccharomyces cerevisiae (ScGH47; Protein Data Bank code 1DL2) and YePL2A superimpose with a root mean square deviation of ~3.1 Å for 310 aligned Cα. Beyond the core of these enzymes, however, there is little structural similarity. The catalytic machinery of ScGH47 is buried within the core of the (α/α)7 barrel in contrast to the position of

FIGURE 1. Enzyme activity of family 2 pectate lyases from Y. enterocolitica. Top left panel, pH optima of YePL2A and YePL2B activity on trigalUA. Top right panel, Metal cofactor depletion studies of YePL2B. Middle left panel, HPAEC product profiling analysis of YePL2A. Middle right panel, YePL2B catalyzed depolymerization of polygalacturonic acid plotted at 30 min and 21 h. Bottom panels, YePL2A and YePL2B activity on trigalUA and polygalacturonic acid (bottom left panel) substrates (bottom right panel) monitored at 232 nm.
the YePL2A active site, which is positioned off-center and above the \( \alpha \)-barrel between the arms of the vice. Also GH47 binds a calcium ion at a noticeably different site than the coordination pocket of the pectate lyase (Fig. 2, C and D). In light of this functional and structural distinction, the phylogeny of this protein fold is difficult to rationalize. Indeed the selective pressures driving the diversification of such unique and specialized enzymes, differing substantially in both substrate specificity and mechanism, are not readily clear. It does suggest, however, that although very few enzymes displaying an \((\alpha/\alpha)\_7\) barrel have been discovered, this fold is a useful scaffold for carbohydrate active enzymes, an observation that has also been noted for the \((\alpha/\alpha)\_6\) toroid family 10 pectate lyases (10).

**Active Site Architecture**—Soaking the inactive YePL2A crystals obtained in the presence of citrate buffer with trigalUA produced a substrate complex with good electron density into which a trigalUA molecule could be unambiguously modeled (Fig. 3A). Analogous experiments with the crystals of the metal-bound form resulted in disintegration of the crystal, which is likely due to the extremely fragile nature of these crystals. The YePL2A active site is positioned at the end of the active cleft, and the intact trigalUA substrate is oriented with its aglycon moiety in contact with the catalytic machinery (Fig. 3A). Analysis of the molecular contacts between YePL2A and trigalUA revealed the presence of three subsites (Fig. 3A); however, this analysis was limited by the substrate size, and quite possibly there are further subsites along the enzyme cleft that would increase its specificity for sugars with a larger degree of polymerization. Subsite +1 (occupied by the leaving group) is primarily composed of the catalytic residues. These include Arg\(^{171}\), which is the putative Brønstead base positioned within 2.9 Å of the aglycon C-5 with satisfactory geometry; and the stabilizing arginine Arg\(^{277}\), which binds both the hydroxyl groups of C-2 and C-3. The uronate group of the sugar residue in the +1 subsite is bound by Glu\(^{130}\) and Glu\(^{515}\) and the amine group of Lys\(^{132}\). At subsite −1 and −2, there are surprisingly few interactions between the substrate and the enzyme, and no detectable salt bridges that may be explained because of the inactivated state of the enzyme. Indeed, it is quite possible that the conformational changes observed upon metal binding and potential for metal coordination with the substrate uronate groups may increase the enzyme specificity for substrate by forming additional interactions. Under these crystallization conditions, hydrogen bonds where detected between Asn\(^{203}\), Lys\(^{132}\), and Tyr\(^{248}\) and the uronate oxygens of the sugar in subsite −1 and also between the O-2 and Arg\(^{530}\) in subsite −2.

In light of these structural data, we next compared the primary structures of YePL2A and YePL2B to determine whether it could explain the differences in activity between the two enzymes. The amino acid alignment demonstrates a reasonably good level of amino acid identity (37%) throughout and the
Structure of a Family 2 Pectate Lyase

The Unique Metal-binding Pocket of Family 2 Pectate Lyases—When crystallized in the presence of citrate buffer (i.e. the native and trigalUA complexed forms), there was no detectable electron density for a metal atom in the predicted coordination site. This observation is explained to be due to the tendency of this buffer to chelate divalent cations and to the fact that the ion may be weakly bound. In another crystallization condition that lacked citrate buffer, however, it was confirmed that the enzyme contains a metal-binding pocket consisting of two histidines, His109 and His172, and one glutamate, Glu130 (Fig. 4A). In the absence of substrate, the metal also coordinates three water ligands.

Although activity recovery assays were inconclusive at directly identifying the optimal cofactor for YePL2A because of experimental limitations, there is strong evidence for transition metal coordination. 1) The amino acids involved in metal coordination in the cytoplasmic pectate lyase PeW from *E. chrysanthemi* (22), the cytoplasmic family 2 *exo*-acting pectate lyase YePL2B from *Y. enterocolitica*, and YePL2A are strictly conserved. The former two enzymes were most proficient in activity recovery assays following Co2+*, Ni2+, and Mn2+ supplementation respectively (Ref. 22 and this study; Fig. 1, bottom left panel). 2) The unique constellation of amino acids within the metal-binding site of YePL2A includes two nitrogen ligands (i.e. histidine residues), which are most appropriate for the coordination of metals such as Mn2+* and Ni2+* (39). Ca2+ coordination is traditionally associated with oxygen-mediated bonds (39) and all other pectate lyases studied to date coordinate Ca2+ in the active site exclusively through oxygen ligands. The presence of three water ligands excludes the possibility of Co2+ utilization (39). 3) The calculated six bond lengths for the atoms ligating the metal atom range between 2.0 and 2.2 Å (Fig. 4A). The positional error of atoms in this structure is on average 0.06 Å. Consequently, some of these distance would overlap with the 2.36 ±(0.10) Å expected for calcium-ligand distances (39). However, when considering the six YePL2A metal-ligand interactions as a group, every single coordination bond is shorter than those expected for Ca2+*. Furthermore, they display a mean distance of 2.10 Å with an estimated error (standard deviation) of ±0.06 Å for this group. This suggests that as a coordination group, the bond lengths are not consistent with those common for Ca2+ binding, indicating that the bound atom is likely not Ca2+* (39).

4) The peak of anomalous signal that overlaps with the electron density of the metal is consistent with the properties of the transition metals, and rules out the possibility of Mg2+ (Mg2+ f* = 0.2 e). 5) To analyze the consistency of B-factor values between the metal atom and amino acids involved in coordination, structural refinements were performed with the metal ion modeled as Ca2+, Mg2+, Co2+, Ni2+, and Mn2+. The average B-factor for the atoms involved in coordinating the metal is 22.4, with a range of 21.7–23.1. This best matches the B-factor of 24.8.

strict conservation of the amino acids predicted to be involved in catalysis (supplemental Fig. 1). The most noticeable difference between the two lyases is an insertion of several amino acids between stands β7 and β8 of YePL2B. When this insertion is mapped to the three-dimensional structure of YePL2A, it is positioned near the end of the active site that interacts with the reducing end of the sugar, where it may function to close off the channel (Fig. 3, B and C). It is easy to envision that such a mechanism may limit the accessibility of the substrate to only +1 and +2 subsites, providing a structural explanation for the exclusive production of digalacturonides by YePL2B (Fig. 1, middle left panel). Active site transformation from an *exo*-acting channel to an *exo*-acting pocket by loop insertions is a well documented phenomenon within glycoside hydrolases, and we have recently reported such a mechanism to explain the structural basis of exopolygalacturonase activity in the family 28 glycoside hydrolases (17); however, less is known about pectate lyases. Unfortunately, further structural analysis of YePL2B by x-ray crystallography has been unsuccessful because of difficulties with protein production and solubility.

**FIGURE 3.** The YePL2A active site and structural basis of *exo*-activity in YePL2B. A, wall-eyed stereo view of trigalacturonic acid in complex with the catalytic residues of YePL2A. The weighted maximum-likelihood (26) Fobs − Fcalc maps (magenta; produced from phases generated by refinements with the trigalUA substrate omitted) for trigalUA is contoured to 1.0σ (0.22 e Å3) and 2.5σ (0.09 e Å3), respectively. The subsites are labeled in red from +1 to −2. B and C, the enzyme is rendered in a solvent-accessible surface (B) (viewing down the active site channel) and ribbon (C) (side view) representation to show the proximity of the inserted loop region colored in red (see supplemental Fig. S1) to the active site (blue).

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4) The peak of anomalous signal that overlaps with the electron density of the metal is consistent with the properties of the transition metals, and rules out the possibility of Mg2+ (Mg2+ f* = 0.2 e). 5) To analyze the consistency of B-factor values between the metal atom and amino acids involved in coordination, structural refinements were performed with the metal ion modeled as Ca2+, Mg2+, Co2+, Ni2+, and Mn2+. The average B-factor for the atoms involved in coordinating the metal is 22.4, with a range of 21.7–23.1. This best matches the B-factor of 24.8.
when the atom is modeled as a Mn$^{2+}$ (other B-factors were: Mg$^{2+}$, 7.9; Ca$^{2+}$, 18.7; Co$^{2+}$, 27.1; and Ni$^{2+}$, 28.3). 5) Although these lyase families are very different in structure, a superimposition of their catalytic centers revealed a remarkable example of functional convergence (10). The YePL2A-trigalUA substrate complex presented here extends this phenomenon to include a third structurally distinct lyase. Although there are a number of unique residues to the family 2 enzyme involved in substrate recognition, there are three residues that closely overlap with amino acids from EcPL1C and CjPL10A that are directly involved in catalysis: Arg$^{171}$, Arg$^{272}$, and Glu$^{130}$ (using YePL2A nomenclature) (Fig. 5D). Importantly, the guanidino group of Arg$^{171}$ aligns with the homologous Bronsted bases from CjPL10A (Arg$^{272}$) and EcPL1C (Arg$^{218}$, the arginine has been reintroduced for reference).

Despite the similarities described above, there are notable differences in the structure of the putative catalytic arginine within YePL2A (Figs. 3A and 5D). First, the catalytic base approaches the substrate from the opposite direction than the family 1 and 10 pectate lyases. In this conformation the imino nitrogen forms a novel interaction with the substrate uronate group, which may assist in stabilizing the reaction intermediate. Second, in a structural overlay including the metal-bound and substrate complex of YePL2A, the metal ion is positioning the catalytic machinery for β-elimination (Fig. 4C). When rendered as a solvent-accessible surface module, the substrate becomes almost completely buried within the active cleft and is suggestive that the enzyme may be processive. This possibility is supported by the accumulation of digalacturonic acid depolymerization (Fig. 1, middle left panel). It is important to mention, however, that this observation may also be influenced by crystal packing effects and solvent because the metal complex crystallized under different conditions. Indeed, in the native and substrate complexes, the crystal forms are generated at pH 5.0, conditions in which the enzyme was inactive. Under optimal conditions (pH 9.6), therefore, the enzyme may adopt a different conformation. In either case it highlights the flexibility in the arm regions of the enzyme.

**Structural Conservation of the β-Elimination Catalytic Machinery**—Previously, β-helix (40, 41) (E. chrysanthemi family 1 pectate lyase, EcPL1C) and (α/α)$_e$ toroid (10) (Cellvibrio japonicus family 10 pectate lyase, CjPL10A) structures in complex with oligogalacturonides have been reported (Fig. 4).
positioned between the two oxygens of the substrate uronate group with equally satisfactory geometries for either atom and at a distinct location when compared with the family 1 and 10 enzymes (Fig. 5D). Although these data must be interpreted with caution because the distances are beyond predicted coordination bond lengths and the metal-bound active form of the enzyme in complex with substrate could not obtained, it does suggest that the metal may form one or even two unique interactions with the substrate in the active form of the enzyme. Although stabilizing interactions for both aglycon carboxylate oxygens have been previously reported (10, 40, 41), in each case it was with two different reacting species.

The other two structurally conserved amino acids play auxiliary roles in catalysis as Arg	extsuperscript{272} (EcPL1C, Arg	extsuperscript{223}; CjPL10A, Arg	extsuperscript{625}) is involved in O-2 and O-3 substrate recognition and Glu	extsuperscript{130} (EcPL1C, Glu	extsuperscript{166}; CjPL10A, Asp	extsuperscript{451}) in metal coordination. The structural and functional group preservation of these residues highlights an underlying principle that the β-elimination of galacto-configuration substrates (Fig. 5E) restricts the flexibility of catalytic site architecture.

**Biological Implications**—There is much speculation as to why pathogens adapted to the colonization of the human gastrointestinal tract have the potential to utilize plant cell wall material as a nutritional source (30, 42) Clearly, the most straightforward explanation is that these enzymes simply scavenge resident pectic products within the colon for catabolism. The lack of extracellular processing enzymes within enteric pathogens suggests that these microorganisms may depend upon preliminary pectin depolymerization catalyzed by other intestinal flora such as Bacterioides sp. In addition, pectin degradation may also be a salvage pathway for energy generation during environmental persistence because Y. enterocolitica has been isolated directly from vegetable food sources (43–46). This possibility has direct or indirect implications for human health because the consumption of such contaminated fruits and vegetables present a ubiquitous vector for food-borne Yersiniosis and other gastrointestinal infectious diseases. In either of these scenarios, inhibiting the enzymes and ligand-binding proteins involved in pectin degradation is therefore a promising opportunity to restrict their nutritional utilization within a human host or prevent the transmission of food-borne illness by hampering their proliferative potential within the primary food source. The novel structures of YePL2A in an apo, metal-bound, and substrate-bound form presented here provide a new platform for research dedicated to such purposes.
Structure of a Family 2 Pectate Lyase

Acknowledgments—We are grateful to Dr. David Vocadlo and Garret Whitworth at Simon Fraser University for assistance with the DIONEX data collection.

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