SUPPORTING INFORMATION

A Unique *Cis*-3-Hydroxy-L-proline Dehydratase in the Enolase Superfamily

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1. Supplementary Information for Enzymology

a. Cloning, Expression and Protein Purification of A0NXQ8 from Labrenzia aggregata IAM 12614.

The gene encoding A0NXQ8 was PCR-amplified from genomic DNA isolated from *Labrenzia aggregata* IAM 12614 using the DNeasy Blood & Tissue Kit (QIAGEN). The PCR reaction (100 μL) contained 100 ng of genomic DNA, 20 μL of 5×GC buffer, 0.2 mM dNTPs, 3.0 μL of DMSO, 2 units of Phusion® High-Fidelity DNA polymerase (New England Biolabs) and 50 pmol of both a forward primer (*Nde*I recognition site; 5'-CTCTGGAGGAGGAACCATATGAAAATTACG-3') and a reverse primer (*Blp*I recognition site; 5'-ATGGGGCTGCCTTTGGCTCAGCCGGCTCAG-3'). The PCR reaction was carried out using a PTC-200 Gradient Thermal Cycle (MJ Research) with the following parameters: 98 °C for 3 minutes followed by 30 total cycles of 98 °C for 30 seconds, gradient temperature range of 50~60 °C for 30 seconds, 72 °C for 60 seconds, and ending with a final extension at 72 °C for 5 minutes. The amplified gene was ligated into the *N*-terminal His_6-tag pET-15b expression vector (plasmid A0NXQ8-pET 15b).

The protein was expressed in *Escherichia coli* strain BL21 (DE3). Transformed cells were grown at 37 °C in 1 L of Luria broth (LB; supplemented with 100 μg/mL ampicillin) to OD_{600} of 0.6 and induced with 0.5 mM isopropyl-*β*-D-thiogalactopyranoside (IPTG). The culture was allowed to grow an additional 20 hr at 20 °C before the cells were harvested by centrifugation. The cells were resuspended in 50 mL of binding buffer (5 mM imidazole, 0.3 M NaCl, 5 mM $MgCl_2$ and 20 mM Tris-HCl, pH 7.9) and lysed by sonication (Fischer Scientific 550 Sonic Dismembrator). The lysate was cleared by centrifugation at 15,000 rpm for 50 minutes at 4 °C. The clarified supernatant containing the His-tagged protein was applied onto a column containing 8 mL of Ni-NTA resin (QIAGEN) previously equilibrated with binding buffer. After equilibration of the Ni-NTA resin with the clarified supernatant on a rocking platform for 40 minutes, the flow-through was discarded. Then, the column was washed with 50 mL of wash buffer I (25 mM imidazole, 0.3 M NaCl, 5 mM $MgCl_2$ and 20 mM Tris-HCl, pH 7.9) followed by 30 mL of wash buffer II (60 mM imidazole, 0.3 M NaCl, 5 mM $MgCl_2$ and 20 mM Tris-HCl, pH 7.9) to elute the unbound proteins. Resin-bound His-tagged protein was eluted with elution buffer (250 mM imidazole, 0.3 M NaCl, 5 mM $MgCl_2$ and 20 mM Tris-HCl, pH 7.9) and

collected in 2 mL fractions. The absorbance at 280 nm of each fraction was measured by a NanoDrop spectrophotometer; the purity of the fractions with strong absorbance at 280 nm (> 1 mg/ml) was checked by SDS-PAGE. After the Ni-column purification, the N-terminal His-tag was removed with thrombin. A stock solution of thrombin from human plasma (lyophilized powder; Sigma) was prepared at a concentration of 1 unit/μL in phosphate buffered saline (PBS) solution. Thrombin was added (3 units/mg of His-tagged protein) and transferred into a 10 mL dialysis cassette (10,000 MWCO, Thermo Scientific) after shaking gently to mix. The cleavage reaction proceeded as it was dialyzed against a solution of 0.3 M NaCl, 5 mM $MgCl_2$, 20 mM Tris-HCl, pH 7.9 and 10 % glycerol at 4 °C for 20 hr. The efficiency of thrombin cleavage reaction was monitored via SDS-PAGE. The cleaved His-tag was removed by first loading the protein solution onto a column containing 4 mL of Ni-NTA resin (QIAGEN) previously equilibrated with storage buffer (150 mM NaCl, 5 mM $MgCl_2$, 20 mM Tris-HCl, pH 7.9, and 10 % glycerol). Then the cleaved protein was eluted with the same storage buffer (150 mM NaCl, 5 mM $MgCl_2$, 20 mM Tris-HCl, pH 7.9, and 10 % glycerol) and concentrated with an Amicon® Ultra centrifugal filter unit (10,000 NMWL, Merck Millipore Ltd.) to a final concentration of ca. 15 mg/mL. The cleaved protein was flash frozen drop wise into liquid nitrogen and stored at -80 °C.

b. Construction, Expression and Protein Purification of K165A and K265A mutants of A0NXQ8.

Megaprimer-based site-directed mutagenesis strategy which involves two rounds of PCR was used for construction of K165A and K265A mutants.

The megaprimer for K165A was constructed using the following 100 μL reaction: 100 ng of wild type plasmid A0NXQ8-pET 15b, 20 μL of 5×GC buffer, 1 mM dNTPs, 2 units of Phusion® High-Fidelity DNA polymerase (New England Biolabs) and 50 pmol of both a forward primer (5'-AAGTTCCAGCTGGCAGTCGGCGGCGATGCCAATGACG-3') and a reverse primer (5'-GCCGCGCACCAGCGTGTTGGGCCCGTCGATCACCTCGTC-3'). The PCR reaction was performed using a PTC-200 Gradient Thermal Cycle (MJ Research) with the following parameters: 98 °C for 5 minutes followed by 30 total cycles of 98 °C for 30 seconds, gradient temperature range of 60~70 °C for 30 seconds, 72 °C for 60 seconds, and ending with a

final extension at 72 °C for 5 minutes. When complete, the amplified product was gel-extracted and kept as K165A megaprimer.

The second round of 100 μL PCR reaction contained 100 ng of wild type plasmid A0NXQ8-pET 15b, 20 μL of 5×GC buffer, 0.3 mM dNTPs, 2 units of Phusion® High-Fidelity DNA polymerase (New England Biolabs), 3.0 μL of DMSO and 29 nM (125 ng) of the K165A megaprimer. The PCR reaction was performed using a PTC-200 Gradient Thermal Cycle (MJ Research) with the following parameters: 98 °C for 5 minutes followed by 20 total cycles of 98 °C for 50 seconds, gradient temperature range of 55~68 °C for 50 seconds, 72 °C for 7 minutes, and ending with a final extension at 72 °C for 7 minutes. When complete, the reactions were pooled together, and 40 units of Dpn I were added and the mixture was incubated at 37 °C for 12 hr. The amplified product was gel-extracted and electroporated into *Escherichia coli* strain XL1-Blue cells. Single colonies were isolated from an overnight culture, and plasmids were isolated from several of these. The presence of the mutation was first investigated by digestion with ApaI (two bands with size as 1.8 kb and 5 kb are observed for the mutant) and then confirmed by DNA sequencing (ACGT, Inc.).

The K265A mutant was similarly constructed using a forward primer (5'-TGCATCAATCTGGCGATCTCCAAGGTCGGCGGCCTGACC-3') and a reverse primer (5'-GTGATGCCGAGCCCGGGCAGGTCGGAGGTGGTCATGCG-3').

The K165A and K265A mutants were purified as described previously for the wild type protein.

c. Cloning, Expression and Protein Purification of D7A0Y2 from Starkeya novella DSM 506.

The gene encoding D7A0Y2 was PCR-amplified from genomic DNA isolated from *Starkeya novella* DSM 506 using the DNeasy Blood & Tissue Kit (QIAGEN). The PCR reaction (100 uL) contained 100 ng of genomic DNA, 20 uL of 5×GC buffer, 0.2 mM dNTPs, 3.0 uL of DMSO, 2 unit of Phusion® High-Fidelity DNA polymerase (New England Biolabs) and 50 pmol of both a forward primer (*Nde*I recognition site; 5'-CAGGAGGGCCTCATATGAAGATCACCGGCATTA-3') and a reverse primer (*Blp*I

recognition site; GTGGATCCCCGGGCTCAGCCCGGGGATGAC-3'). The protein was purified as described previously for A0NXQ8.

d. Cloning, Expression and Protein Purification of D7A0Y3 and D7A0Y1 from Starkeya novella DSM 506.

The gene encoding D7A0Y3 was PCR-amplified from genomic DNA isolated from *Starkeya novella* DSM 506 as described in the D7A0Y2 section using a forward primer (*Nde*I recognition site; 5'-GACGAGTGACCCATATGGCGCGACACAGC-3') and a reverse primer (*Blp*I recognition site; 5'-CATGAGCCGGGCTCAGCAGGATGAGGTTG-3'). The amplified gene was ligated into the *N*-terminal His_6-tag pET-15b expression vector (plasmid D7A0Y3-pET 15b). The protein was expressed in *Escherichia coli* strain BL21 (DE3). Transformed cells were grown at 37 °C in 2 L of Luria broth (LB; supplemented with 100 μg/mL ampicillin) to OD_{600} of 0.6 and induced with 0.5 mM isopropyl-*β*-D-thiogalactopyranoside (IPTG). The culture was allowed to grow an additional 20 hr at 20 °C before the cells were harvested by centrifugation. The cells were resuspended in 80 mL of binding buffer (5 mM imidazole, 0.3 M NaCl, and 20 mM Tris-HCl, pH 7.9) and lysed by sonication (Fischer Scientific 550 Sonic Dismembrator). The lysate was cleared by centrifugation at 15,000 rpm for 50 minutes at 4 °C. The clarified supernatant containing the His-tagged protein was applied onto a column containing 8 mL of Ni-NTA resin (QIAGEN) previously equilibrated with binding buffer. After equilibration of the Ni-NTA resin with the clarified supernatant on a rocking platform for 40 minutes, the flow-through was discarded. Then, the column was washed with 50 mL of wash buffer I (25 mM imidazole, 0.3 M NaCl, and 20 mM Tris-HCl, pH 7.9) followed by 30 mL of wash buffer II (60 mM imidazole, 0.3 M NaCl, and 20 mM Tris-HCl, pH 7.9) to elute the unbound proteins. Resin-bound His-tagged protein was eluted with elution buffer (250 mM imidazole, 0.3 M NaCl, and 20 mM Tris-HCl, pH 7.9) and collected in 2 mL fractions. The absorbance at 280 nm of each fraction was measured by a NanoDrop spectrophotometer; the purity of the fractions with strong absorbance at 280 nm (> 1 mg/ml) was checked by SDS-PAGE. The purified fractions were pooled and concentrated with an Amicon® Ultra centrifugal filter unit (10,000 NMWL, Merck Millipore Ltd.) to a final volume of 2.5 mL. Imidazole and excess salt were removed by passing the protein solution through a PD 10 desalting column containing 8.3 mL of Sephadex™ G-25 medium (GE Healthcare) previously equilibrated with storage buffer (150 mM NaCl, 20 mM

Tris-HCl, pH 7.9, 2 mM DTT and 10 % glycerol). The protein was eluted with 3.5 mL of storage buffer and flash-frozen into liquid nitrogen and stored at -80 °C.

The gene encoding D7A0Y1 was PCR-amplified from genomic DNA isolated from *Starkeya novella* DSM 506 as described in the D7A0Y3 section using a forward primer (*Nde*I recognition site; 5'-GAAGAGGTTCCCATATGCGCTCATCCAAGG-3') and a reverse primer (*Blp*I recognition site; 5'-CTAAGGCGGACGGGCTCAGCCACCGTCAGTC-3'). The amplified gene was ligated into the *N*-terminal His_6-tag pET-15b expression vector (plasmid D7A0Y1-pET 15b). The protein was expressed in *Escherichia coli* strain BL21 (DE3) and purified using the same protocol as described for D7A0Y3.

e. In vitro Activity Measurements for A0NXQ8, the K165A and K265A mutants of A0NXQ8, and D7A0Y2.

Screening of proline analogue library by ^{1}H NMR for A0NXQ8. The reaction mixture (800 μL) containing 1 μM of purified enzyme, 5 mM $MgCl_2$, 25 mM sodium phosphate buffer, pD 8.0, and 5 mM substrate in D_2O was incubated at 30 °C for 16 hrs. The ^{1}H NMR spectrum then was analyzed for deuterium incorporation or dehydration. The proline analogue library is shown in Figure S1.

The *in vitro* activities of the K165A and K265A mutants of A0NXQ8 and D7A0Y2 were tested as described above.

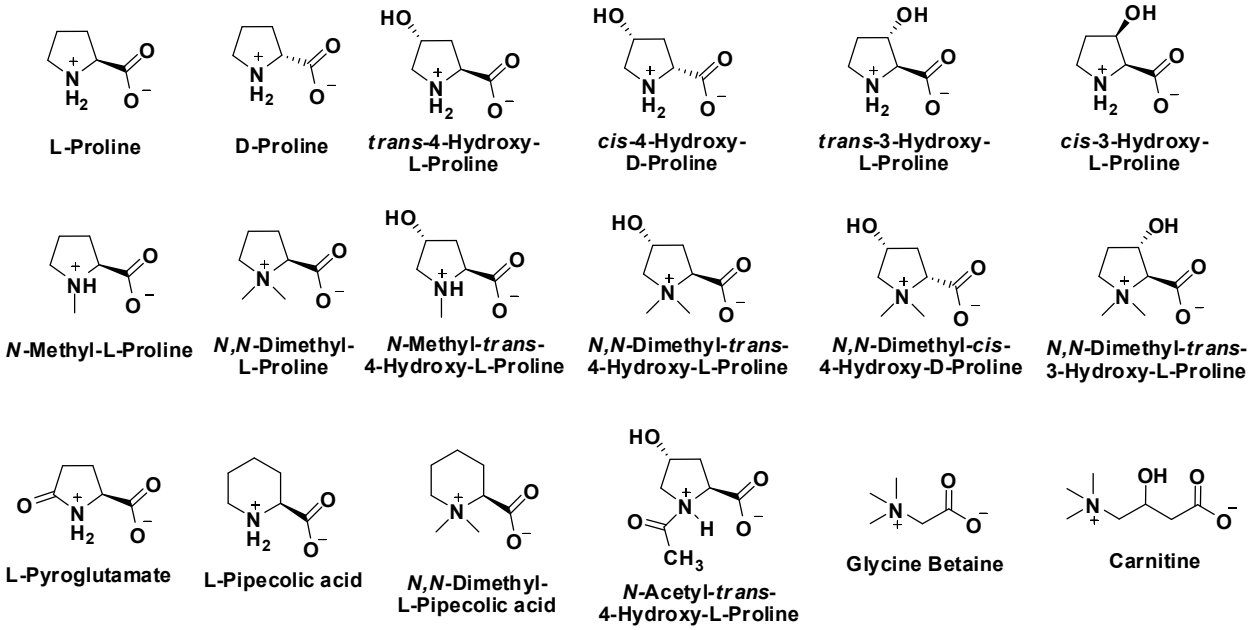

Figure S1. Proline Analogue Library.

Activity of the K165A and K265A mutants using *trans*-3-hydroxy-D-proline by ^{1}H NMR

The reaction mixture (3 mL) containing 0.5 μM of purified A0NXQ8, 5 mM $MgCl_2$, 50 mM sodium phosphate buffer, pH 8.0, and 20 mM *cis*-3-hydroxy-L-proline in H_2O was incubated at 30 °C for 3 hrs. A parallel reaction in D_2O was performed to monitor the reaction progress by ^{1}H NMR spectroscopy so that reaction in H_2O could be stopped when a 1:1:1 mixture of *cis*-3-hydroxy-L-proline, *trans*-3-hydroxy-D-proline and Pyr2C was reached (see Figure 4). The enzyme was removed by filtration through Amicon centrifugal filters (10,000 MWCO, Merck, Millipore Ltd.), and the filtrate was lyophilized. The residue was dissolved in 3 mL D_2O, and the ^{1}H NMR spectrum was analyzed. The dehydration product Pyr2C decomposed upon lyophilization. The ratio of *cis*-3-hydroxy-L-proline to *trans*-3-hydroxy-D-proline is ~1:1 based on the integral of the α-proton on carbon-2.

Then, the 3 mL reaction mixture was split into three equal portions. The first portion was used as the control reaction; 1 μM of K165A mutant was added to the second portion, and 1 μM of K265A mutant was added to the third portion. The three reactions were incubated at 30 °C for 60 hrs and monitored by ^{1}H NMR. Neither mutant showed any activity for either dehydration or exchange of α-proton with solvent deuterium (Figure S2).

To determine whether the decomposed Pyr2C inhibited the reactions, 1 μM of wild-type A0NXQ8 was added to the control reaction and incubated at 30 °C for 16 hrs. No inhibition was observed because both *cis*-3-hydroxy-L-proline and *trans*-3-hydroxy-D-proline were transformed into Pyr2C based on analysis of the ^{1}H NMR spectrum.

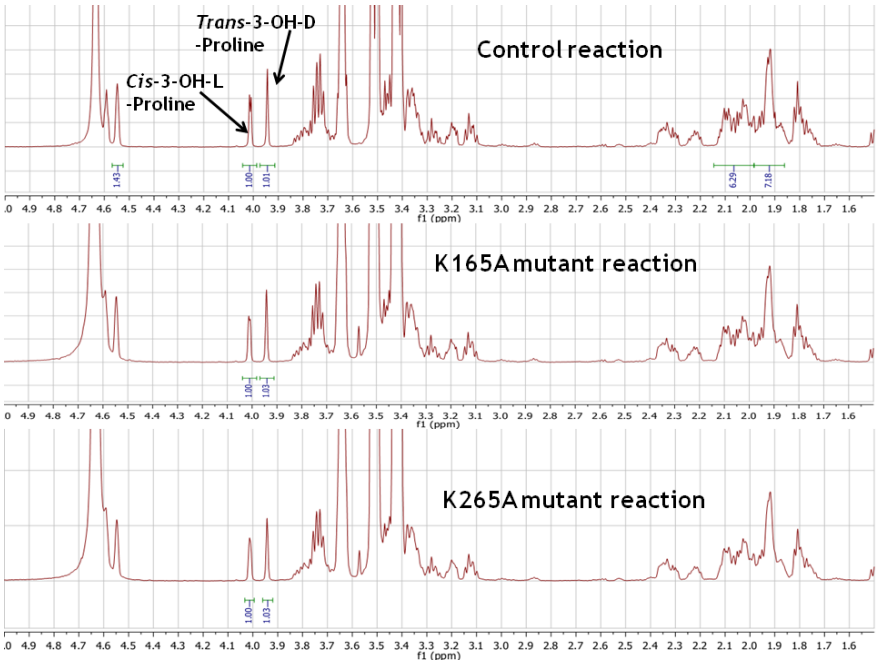

Figure S2. ^{1}H NMR spectra for *t*3DHyp incubated with K165A and K265A mutants.

f. Kinetic Measurements for A0NXQ8.

Polarimetric assay for A0NXQ8. The change of optical rotation was measured in a Jasco P-1010 polarimeter at 25 °C using a 10-cm path length cuvette and a mercury line filter (405 nm). The assays (1.5 mL) contained variable concentrations of substrate, 10 mM $MgCl_2$, 50 mM Tris-Cl buffer, pH 8.0, and purified enzyme.

^{1}H NMR kinetic assay for A0NXQ8. The progress of the reaction was monitored by continuous ^{1}H NMR assay (Agilent 600MHz NMR) based on the change of intensities of the signals associated with *cis*-3-OH-L-proline, *trans*-3-hydroxy-D-proline and Pyr2C.

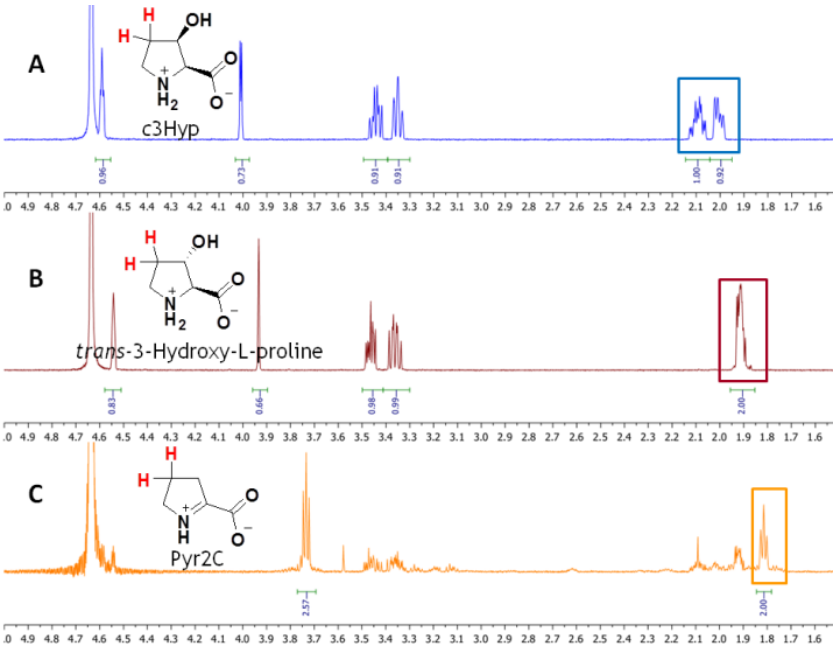

Figure S3. ^{1}H NMR spectra for *c*3LHypD catalyzed reaction (The boxed peaks are corresponding to the red hydrogens displayed in the chemical structures, all the spectra are taken in 25mM sodium phosphate buffer, pD 8.0, and 5 mM $MgCl_2$ in D_2O). (A) ^{1}H NMR spectra of *c*3LHyp; (B) ^{1}H NMR spectra of *trans*-3-OH-L-Proline (identical to *trans*-3-OH-D-Proline); (C) ^{1}H NMR spectra of *c*3LHyp substrate incubated with purified A0NXQ8 at 30 °C for 16h.

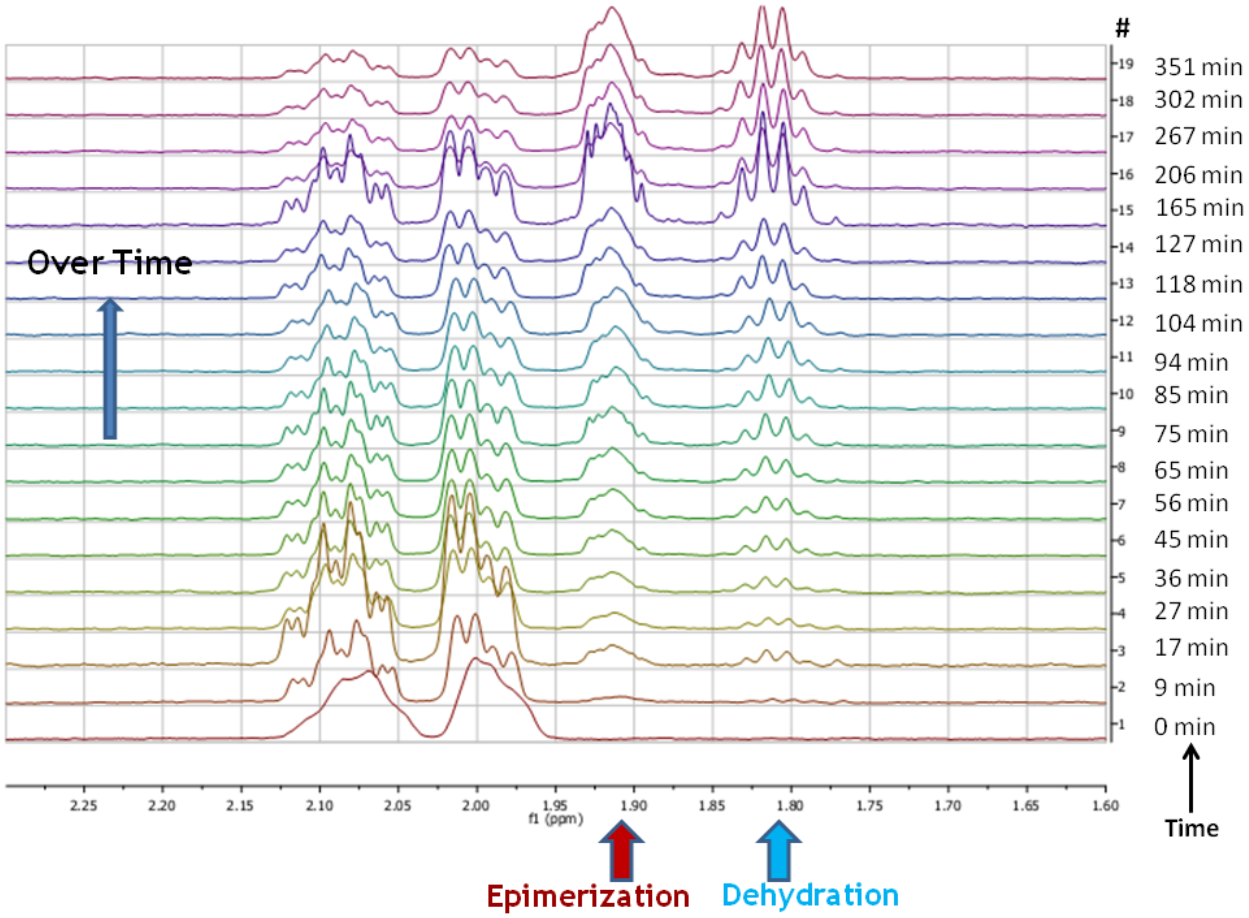

Figure S4. A stack-plot of the time-dependent ^{1}H NMR spectra of A0NXQ8 catalyzed reaction. As the reaction proceeds, the intensities of the signals associated with *c*3LHyp decrease as the intensities of the signals associated with *trans*-3-hydroxy-D-proline and Pyr2C increase concurrently.

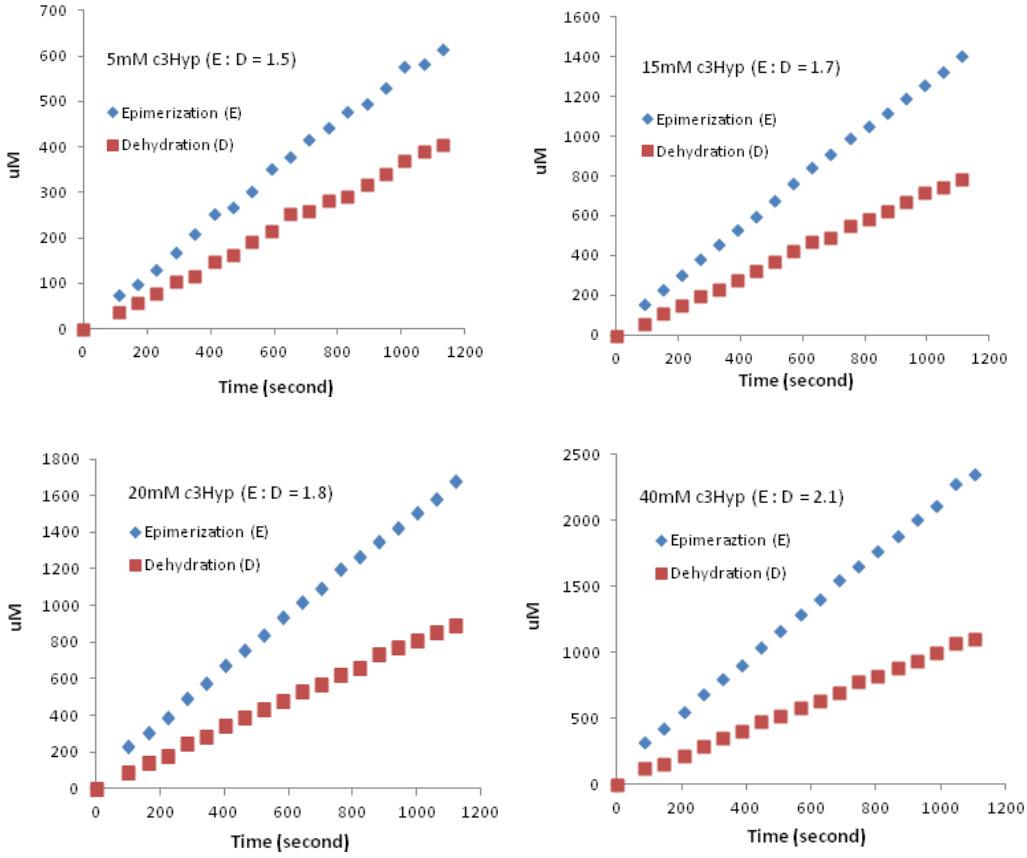

Figure S5. The relative rate of epimerization and dehydration of A0NXQ8 catalyzed reaction was determined by the continuous ^{1}H NMR assay. The assays (0.8 mL) contained variable concentrations of the substrate (5 mM, 15 mM, 20 mM and 40 mM), 10 mM $MgCl_2$, 50 mM sodium phosphate buffer, pH 8.0 and purified enzyme at 25 °C.

g. *In vitro Activity Measurements for D7A0Y3 and D7A0Y1.*

^{1}H NMR assay for D7A0Y3 and D7A0Y1. The reaction mixture containing 1 μM of purified enzyme, 25 mM sodium phosphate buffer, pD 8.0, and 5 mM substrate in a total volume of 800 μL was incubated at 30 °C for 16 hr. The ^{1}H NMR spectra were analyzed for deuterium incorporation or dehydration. The substrates screened for D7A0Y3 and D7A0Y1 include L-proline, *trans*-4-OH-L-proline, *trans*-3-OH-L-proline and *cis*-3-OH-L-proline.

h. In vitro Activity and Kinetic Measurements for D7A0Y0.

^{1}H NMR assay for Δ^1-pyrroline-2-carboxylate reductase activity. The reaction mixture containing 10 mM of Δ^1-pyrroline-2-carboxylate, 1 μM enzyme, 0.16 mM NADPH, 25 mM sodium phosphate buffer, pD 8.0, 1 U/mL alcohol dehydrogenase ($NADP^+$-dependent from *Thermoanaerobium brockii*, Sigma) and 80 μL isopropanol in a total volume of 800 μl was incubated at 30 °C for 16 hr. The solvent was removed by lyophilization; 800 μL D_2O was added, and the ^{1}H NMR spectrum was recorded.

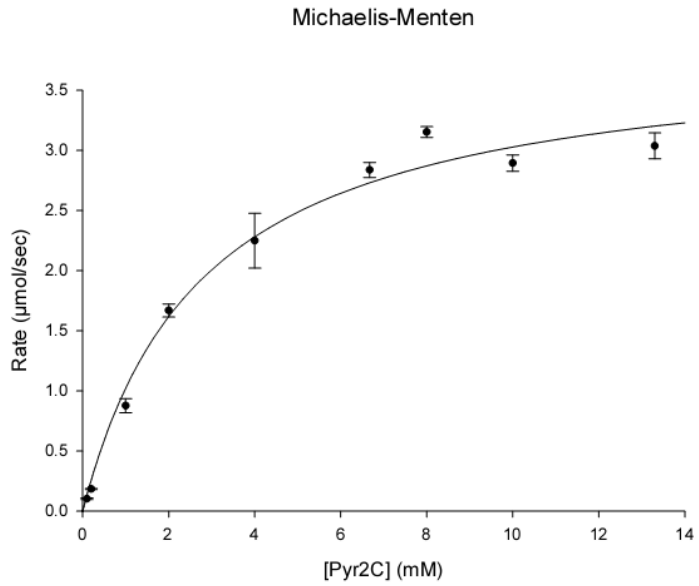

Figure S6. Δ^1-Pyrroline-2-carboxylate reductase assays were carried out by measuring the decrease in the absorbance of NADPH at 340 nm with a Cary 300 Bio UV-Visible spectrophotometer (Varian) at 25 °C. The reaction mixture contained variable concentrations of the Δ^1-pyrroline-2-carboxylate, 50 mM Tris-HCl buffer, pH 7.6, 0.16 mM NADPH, and 0.15 μM enzyme in a final volume of 300 μL. Data were fit to the Michaelis-Menten equation.

2. Supplementary Information for Microbiology

L. aggregata Strain Growth Conditions

L. aggregata was grown in minimal medium [K_2HPO_4 (17.0 g/L), $(NH_4)_2SO_4$ (2.5 g/L), NaCl (2.0 g/L), $MgSO_4.7H_20$ (1.0 g/L), $CaCl_2.2H_2O$ (0.15g/L), $FeSO_4.7H_20$ (0.01 g/L), $ZnSO_4.H_20$ (2.0 g/L), $CuSO_4.5H_2O$ (0.4 g/L), H_3BO_3 (0.1 g/L), 30 g/L sea salts (Sigma), Thiamine (0.01 g/L), nicotinic acid (0.01 g/L) biotin (10 nM)] supplemented with 20 mM glucose or *cis*-3-hydroxy-L-proline or *trans*-3-hydroxy-L-proline or *trans*-4-hydroxy-L-proline as a carbon source.

Gene Expression analysis.

Cell preparation. The bacterial strain was grown in 5 mL of minimal medium with 20 mM glucose as carbon source to an OD_{600} of 0.4. The cells were pelleted by centrifugation (4,000 x g, 10 min, 4 °C). The cell pellet was washed twice and resuspended in 5 mL of minimal medium lacking a carbon source. The cultures were divided into two 2.5 ml aliquots. Glucose was added to one aliquot and *cis*-3-hydroxy-L-proline or *trans*-3-hydroxy-L-proline or *trans*-4-hydroxy-L-proline was added to the other as carbon source followed by aerobic growth at 30 °C for 3 hrs prior to cell harvest.

RNA Sample preparation. RNA samples were prepared and qRT PCR was performed as described in previous published paper (1). Efficiency of all the primers used in qRT PCR was calculated as 96% $\pm$ 2%. The gene expression data were expressed as Cp or cross point values. The 16S rRNA was used as a reference gene. The data was analysed by $2^{-\Delta\Delta CT}$ (Livak) method (2). The reported values are the averages of five trials. Primer sequences are provided in the **Table S2**.

Table S1. Transcriptional analysis: The expression profiles in cells grown with *cis*-3-hydroxy-L-proline, *trans*-3-hydroxy-L-proline and *trans*-4-hydroxy-L-proline are compared to cells grown with glucose as carbon source. Bacterial strain was cultured in minimal medium containing 20 mM glucose sole carbon source and gene expression was compared with the cells grown in minimal media with different carbon sources, i.e. *cis*-3-hydroxy-L-proline, *trans*-3-hydroxy-L-proline and *trans*-4-hydroxy-L-proline. The identity of gene is indicated by the UniProt ID. Assays were performed in triplicates; data are averages with standard deviation. The average ratio is the fold difference compared to cells grown with glucose as a carbon source. P value < 0.05.

Organism/UniProt ID	***cis*-3-hydroxy-L-proline**	***trans*-3-hydroxy-L-proline**	***trans*-4-hydroxy-L-proline**
***L. aggregata* IAM12614**			
A0NXQ8 (*c*3LHypD)	**10.5 ± 1.2**	**15 ± 2**	**6 ± 1**
A0NXQ9 (*t*3LHypD)	**9.0 ± 1.0**	**12 ± 2**	**6 ± 2**
A0NXQ7 (4HypE)	**4.1 ± 0.5**	**5 ± 2**	**22 ± 2**
DHDPS	**5.2 ± 0.5**	**4 ± 1**	**18 ± 2**

Table S2. Primers used in this study

Primer Name	Primer Seq 5' to 3'
LA16SRTF	TGGTGGGGTAAAGGCCTAC
LA16SRTR	TGGCTGATCATCCTCTCAGAC
A0NXQ8 LARTF	CGCTCAACATCGGTGAGAT
A0NXQ8 LARTR	CCCCAAGCAACGTATAAAGC
A0NXQ9 LartF	TGTTGAAGACGAGGCCAAG
A0NXQ9 LartR	AAAAGCCGAGCTGTTCGTT
A0NXQ7 LartF	CGCGTAATCGACAGCCATA
A0NXQ7 LartR	GGCACAGAAATCGAGATGCT
LADHDPSRTF	CCGAAGGAAGTTCTGCATCT
LADHDPSRTR	TTCCGCATGACCTTCTGC

3. Supplementary Information for Crystallization and Structure Determination

Crystals of A0NXQ8 were grown by sitting drop vapour diffusion in 96-well INTELLI-plates (Art Robbins Instruments, Sunnyvale, CA) at room temperature (~294 °K). Protein (17 mg/mL, 5 mM $MgCl_2$, 20mM Tris-HCl buffer pH 7.9, and 5% glycerol) was mixed with an equal volume (1 µl) of precipitant solution utilizing a PHOENIX crystallization robot (Art Robbins Instruments) and four commercial crystallization screens: the MCSG screens 1, 2 and 4 (Microlytic, Woburn, MA) and the MIDAS screen (Emerald BioSystems, Bainbridge Island, WA). Crystals grew as small rods (0.02 x 0.02 x 0.1 mm) after 2 months with 40% w/v pentaerythritol propoxylate (17/8 PO/OH), 0.2 M $MgCl_2$, 0.1 M MES-NaOH, pH 5.5 as the precipitant. Crystals were cooled to 100 °K by plunging directly into liquid N_2 with the reservoir solution as the cryoprotectant.

X-ray diffraction data at 100 °K were collected from a single crystal on beamline 31-ID (LRL-CAT, Advanced Photon Source, Argonne National Laboratory, IL, USA) using a wavelength of 0.9793 Å. Data were integrated with MOSFLM (3) and scaled with SCALA (4). Molecular replacement within the program AMORE (5) was utilized to phase the structure with two subunits of the PDB deposition 2PMQ as a search model (33% sequence identity). Iterative rounds of manual rebuilding in the molecular graphics program COOT (6) with refinement in PHENIX (7) were performed to finalize the structure determination and the molecular geometry was checked with MOLPROBITY (8). The final model consists of eight subunits in the asymmetric unit with an octameric oligomeric arrangement consistent with the known oligomeric structure of many enolase family members. Each subunit has a well resolved Mg ion in the active site coordinated (O-Mg dist. 2.0-2.1 Å) by three carboxylate sidechains (Asp193,

Asp241, Glu218) and three waters in a square bipyramidal geometry consistent with the known geometry of Mg ions bound to enolase family members. Density for the flexible 20s' and 50s' loops was unresolvable in several subunits (see Table **S3** for details).

Table S3. Data Collection and Refinement Statistics[a]

Protein UniProt ID	A0NXQ8
Organism	*Labrenzia aggregata*
PDBID	4MGG
DIFFRACTION DATA STATISTICS	
Space Group	$P2_12_12_1$
Unit Cell (Å , °)	a=110.3 b=154.9 c=182.0
Resolution (Å)	40.8-2.2 (2.32-2.20)
Completeness (%)	99.9 (99.8)
Redundancy	7.3 (7.1)
Mean(I)/sd(I)	9.3 (2.8)
R_{sym}	0.184 (0.804)
REFINEMENT STATISTICS	
Resolution (Å)	40.8-2.20 (2.23-2.20)
Unique reflections	157964 (5173)
R_{cryst} (%)	15.0 (21.4)
R_{free} (%, 5% of data)	21.6 (30.1)
Residues In Model [Expected]	[1-367] A(-1)-367, B1-53, B60-367, C1-367, D0-53, D61-367, E0-53, E61-367, F0-53, F60-367, G1-17, G29-54, G60-367, H0-53, H60-367
Residues / Waters / Atoms total	2894 / 1878 / 26755
Bfactor Protein/Waters/Ligand (Mg^{2+})	27.6 / 31.1 / 22.4
RMSD Bond Lengths (Å) / Angles (°)	0.014 / 1.38
Ramachandran Favored / Outliers (%)	97.4 / 0.3
Clashscore[b]	3.6 (99^{th} pctl.)
Overall score[b]	1.5 (98^{th} pctl.)

[a] Data in parenthesis is for the highest resolution bin

[b] Scores are ranked according to structures of similar resolution as formulated in MOLPROBITY

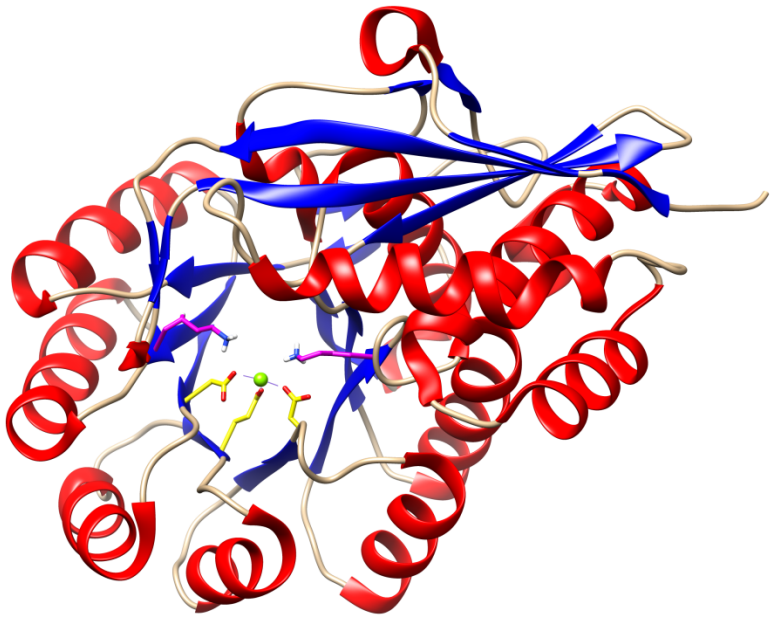

Figure S7. Experimentally determined crystal structure of A0NXQ8 with bound Mg^{2+}. The Mg^{2+} is represented by the green sphere. The ligands for $Mg^{2+,}$ Asp193, Glu218 and Asp24, are highlighted in yellow; the conserved acid/base catalysts, Lys165 and Lys 265, are highlighted in magenta.

References

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