SUPPORTING INFORMATION

Deracemization and Stereoinversion of α-Amino Acids by L-Amino Acid Deaminase

Elena Rosini[a,b,*], Roberta Melis[a,b], Gianluca Molla[a,b], Davide Tessaro[b,c], and Loredano Pollegioni[a,b]

[a] Department of Biotechnology and Life Sciences, Università degli Studi dell'Insubria, via J.H. Dunant 3, 21100 Varese, Italy; e-mail:elena.rosini@uninsubria.it

[b] The Protein Factory, Politecnico di Milano and Università degli Studi dell'Insubria, via Mancinelli 7, 20131 Milano, Italy

[c] Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, p.zza Leonardo da Vinci 32, 20133 Milano, Italy

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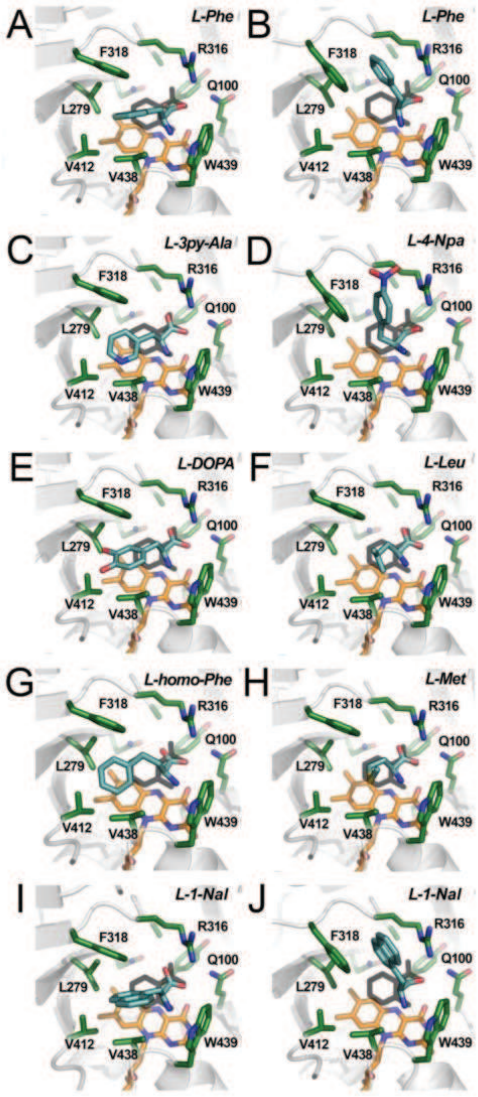
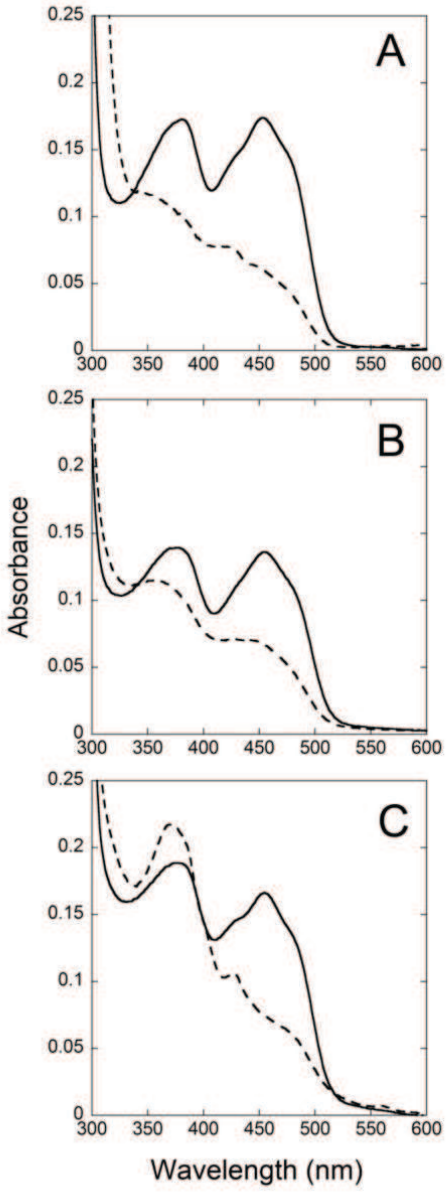
Docking analysis

Figure S1. Substrate binding at the active site of PmaLAAD. Different substrates have been docked into the active site of PmaLAAD (PDB code: 5fjn). The ligand 2-aminobenzoate which is present into the crystallographic structure is shown in light gray. Active site residues belonging to the first shell are represented in green while the substrate is represented in cyan. In the case of the ligands L-Phe and L-1-Nal two alternative substrate conformations are shown. The FAD cofactor is shown in yellow.



Assay of PmaLAAD activity on poor substrates

Figure S2. Spectral analysis of 10 µM PmaLAAD (continuous line) to which 0.25 mM L-4-methoxy-phenylglycine (A), 0.25 mM L-4-methyl-phenylglycine (B), or 10 mM L-penicillamine (C) under anaerobic conditions (dashed line) was added.



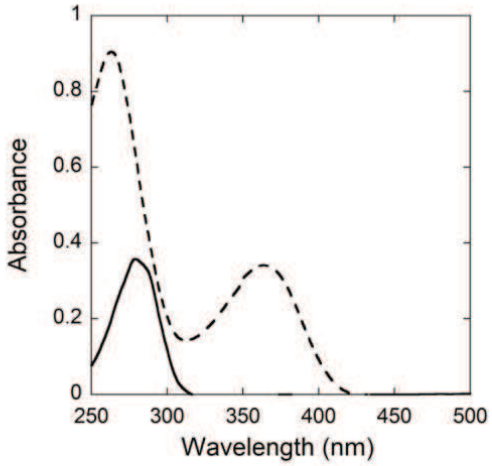
S3

Set up of bioconversions of different natural and synthetic L-amino acids

With the ultimate aim of identifying the best reaction conditions for L-amino acid bioconversions, the time course of the deamination of 25 mM L-Phe was investigated using 0.1 mg/mL (corresponding to 0.3 U/mL) of PmaLAAD at 25 or 37 °C. No significant decrease in enzyme activity was observed after 8 h of incubation at either of the temperatures tested. Nevertheless, after 18 h of incubation, 50% of the initial activity value was recovered at 37 °C while full activity was retained by incubating the enzyme at 25 °C. Accordingly, all bioconversion reactions were conducted at 25 °C and pH 7.5 (the only exception was L-DOPA), i.e., the value at which PmaLAAD shows maximal activity.

A preliminary investigation of the deaminase activity on different natural and synthetic L-amino acids was performed spectrophotometrically measuring the formation of the corresponding ketone. As shown in Figure S3, an increase in the absorbance at 360 nm was apparent following the oxidation of 1.2 mM D,L-1-Nal by PmaLAAD (0.1 mg/mL). This assay was used to identify the optimal initial concentration for each substrate.

Figure S3. Spectral analysis of the reaction mixture containing D,L-1-Nal before (continuous line) and 6 h after (dashed line) adding 0.1 mg PmaLAAD/mL. To both samples 3 N NaOH was added to stop the reaction and allow the color to develop.



Next, the bioconversion results were verified by measuring the residual L-amino acid through the ninhydrin method. Finally, under optimized reaction conditions, the time course of conversion of different L-amino acids (Table S1) and the resolution of different racemic mixtures by the deaminase enzyme were analyzed by HPLC: the products of the bioconversion reactions were separated and quantified by reverse-phase HPLC and the enantiomeric purity was evaluated by chiral HPLC.

Table S1. Enzymatic bioconversion of different L-amino acids by PmaLAAD.

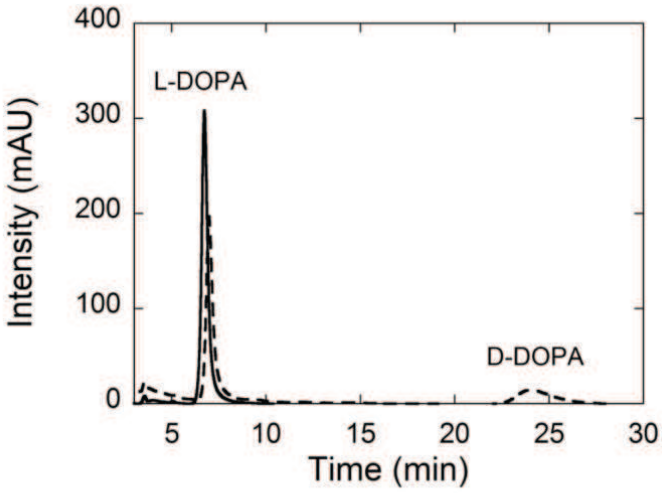
Compound	Initial concentration (mM)	Conversion yield of L-enantiomer (%)	Product formation at 90% conversion (μmol/min·mg enzyme)
L-Phe[a)]	25	> 95	1.27 (2.92)[d)]
L-4-Npa[a)]	12.5	> 99	6.80
L-DOPA[b,c)]	25	~ 90	0.97

The bioconversion was carried out at 25 °C, pH 7.5, employing 0.1 mg/mL enzyme (corresponding to 0.3 U/mL on L-Phe) at air saturation, under optimized conditions. The products of the bioconversions were analyzed by HPLC with a Luna 5 μm C18(2) column. HPLC conditions: [a)]70% A, 30% B; detection at 210 nm; [b)]95% A, 5% B; detection at 280 nm. A = H_2O (0.1% TFA), B = MeOH (0.1% TFA). [c)]The bioconversion was performed at pH 7.0 because of a higher substrate stability. [d)]The bioconversion was performed by equilibrating the reaction mixture with pure oxygen.

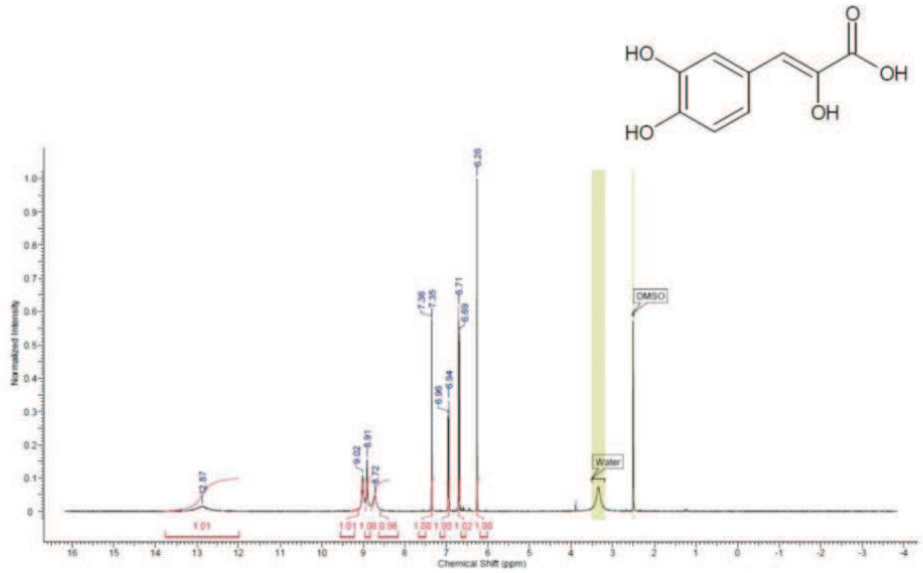
Chemoenzymatic stereoinversion of L-DOPA

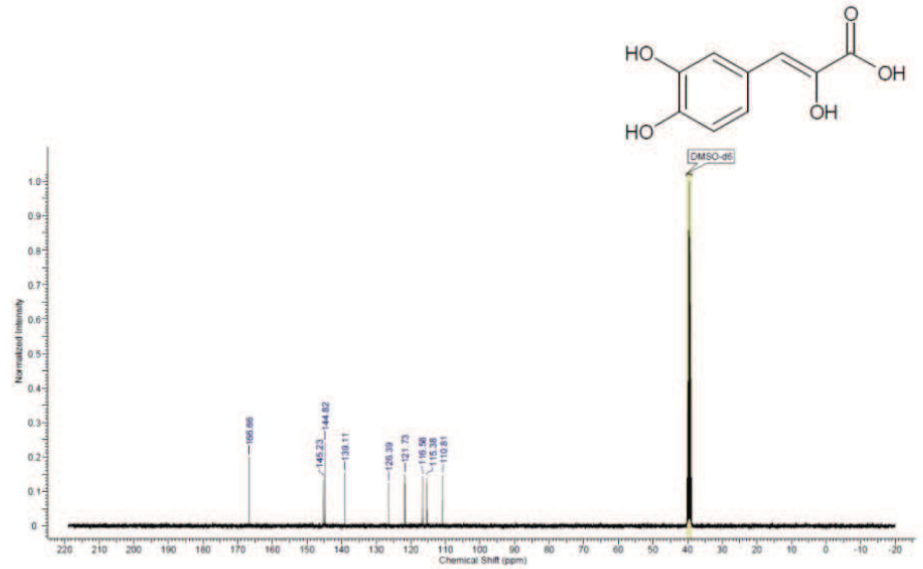
The generation of D-DOPA from the corresponding L-enantiomer was established by coupling the deamination of the L-amino acid to the corresponding achiral imino acid and its nonselective *in situ* reduction by the borane *tert*-butylamine complex (Scheme 1B). In the presence of 25 mM L-DOPA, production of the corresponding D-enantiomer reached a plateau after 24 h, corresponding to ~ 20% amino acid stereoinversion (Figure S4). For the achiral imino acid nonselective reduction borane triethylamine complex and borane morpholine complex were also used: an overall conversion yield of ~ 8% and 4.5% was obtained, respectively. Indeed, in presence of the bis(benzonitrile)palladium(II) chloride as hydrogen transfer catalyst, no D-enantiomer production was observed. A lower amount of the substrate or a higher number of molar equivalents of the reducing agent negatively affected the overall yield of reduction.

Figure S4. Stereoinversion of L-DOPA by PmaLAAD and nonselective reduction (see Scheme 1B). HPLC chromatogram of 25 mM L-DOPA before (continuous line) and 24 h after adding PmaLAAD and 125 mM borane *tert*-butylamine complex reducing agent (dashed line), at 25 °C and pH 7.0.



^{1}H and ^{13}C NMR spectra of 3-(3,4-dihydroxyphenyl)-2-oxopropanoic acid





^{1}H and ^{13}C NMR spectra of D-4-Npa obtained from chemoenzymatic steroinversion