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Egon Heuson,[a] Franck Charmantray,[a] Jean-Louis Petit,[b] Véronique de Berardinis,[b] and Thierry Gefflaut [a]*

[a] Université Clermont Auvergne, CNRS, SIGMA Clermont, ICCF, F-63000 Clermont-Ferrand, France.
Fax: (+33) 473407717; Phone: (+33) 473407866; E-mail: thierry.gefflaut@uca.fr
[b] Génomique métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Univ Paris-Saclay, 91057 Evry, France

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Abstract. Enzymatic transamination is a useful method for the green and highly enantioselective synthesis of chiral amines and non-canonical amino acids which are of major importance as intermediates in medicinal chemistry. However, transamination reactions are usually reversible and synthetic applications of transaminases often require the implementation of an equilibrium shift strategy. Herein, we report a highly effective approach using glutamine as smart amine donor. This amino acid is converted upon transamination into 2-oxoglutaramate which undergoes a fast cyclisation displacing the transamination equilibrium. We have developed a new activity assay in order to identify transaminases from biodiversity able to convert various α-keto acids into valuable amino acids of L- or D-series in the presence of glutamine as amine donor. Discovered transaminases were then used to prepare in high yield and with high enantioselectivity three amino acids of pharmaceutical importance, homophenylalanine, homoalanine and *tert*-leucine by simply using a nearly stoichiometric amount of glutamine as amine donor.

Keywords: Amination; Amino acids; Biocatalysis; Transferases

Introduction

The tremendous structural diversity of biomolecules notably relies on the combination of 20 standard amino acids in proteins. Moreover, the chemists ability of preparing an unlimited variety of non-proteinogenic (also named "non-canonical" or "unusual") amino acids still expand the chemical space of peptides and peptidomimetics which are of increasing importance in modern drug discovery.[1]

Among the numerous synthetic routes towards amino acids,[2] bioprocesses and biocatalytic methods benefit from the high enantioselectivity of enzymes while meeting the criteria of green and sustainable chemistry.[3] Among various enzyme families used for amino acids synthesis, transaminases (TA) have been extensively studied in the large-scale preparation of unusual L- or D-α-amino acids as well as a variety of chiral amines.[4]

TA catalyze the pyridoxal 5'-phosphate-dependent reversible transfer of the amino group from an amine donor substrate onto an acceptor carbonyle substrate. The TA family is highly diverse, comprising many ubiquitous L-*α*-TA involved in proteinogenic L-amino acids metabolism. Moreover, some microbial D-*α*-TA are specific for amino acids of the D-series whereas amine-TA (ATA also called *ω*-TA) are active with a variety of amines lacking the *α*-carboxylic acid moiety.[5] All L- or D-*α*-TA studied to date, proved to be highly enantioselective catalysts and most of them displayed relaxed substrate specificity. High turnovers and no requirement for external cofactor recycling are additional features making TA valuable biocatalysts to access a variety of unusual amino acids.

However, the major drawback of transamination reactions lies in their equilibrium constants, often close to unity and resulting in limited yields and purification issues.[6] To overcome these thermodynamic limitations, an equilibrium shift strategy is needed. It usually consists in the removal or transformation of the carbonyl coproduct formed from the amine donor substrate. One striking example is the removal under reduced pressure of acetone formed from isopropylamine used as amine donor for particular ATA.[7] This approach was notably adopted in a synthetic process for the antidiabetic drug sitagliptine.[8] However only few ATA are active with isopropylamine and this strategy is not applicable to α-TA which are highly specific for α-amino acids.

Alternatively, equilibrium shift was often achieved through auxiliary enzymatic reactions as exemplified by the lactic-dehydrogenase catalyzed reduction,[9] acetolactate-synthase catalyzed autocondensation[10] or pyruvate-decarboxylase catalyzed decarboxylation[11] of pyruvic acid (Pyr) formed from alanine (Ala) used as amine donor.

In a simpler way, a transamination reaction can be displaced through the use of smart amine donors. These so-called substrates are indeed converted upon transamination into unstable carbonyl coproducts that undergo a spontaneous transformation affording the needed equilibrium shift. For example, aspartic acid (Asp) or its analogue cysteine sulfinic acid (CSA) used as amine donors are converted into β-keto carboxylic or sulfinic acids which lose carbon or sulfur dioxide to give pyruvic acid (Pyr). This strategy was however limited to particular TA like Aspartate–TA or Tyrosine-TA, that are active with Asp or CSA as amine donor and unable to convert Pyr, formed from these substrates, into Ala.[12]

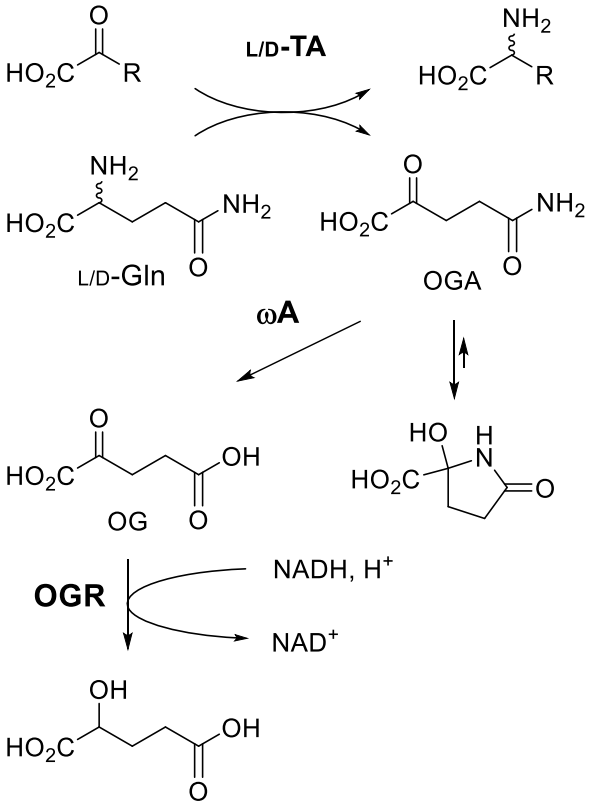
Another class of smart amine donors is defined by compounds bearing an extra amino group able to react with the carbonyl generated upon transamination to give a cyclic imine. Therefore, the α,ε-diamino acid lysine was used as irreversible amine donor with a genetically modified AspTA to produce L-homophenylalanine (Hfe)[13] whereas ornithine was used in an equilibrium shifted process using an ornithine-ω-TA as auxiliary enzyme.[14] More recently, several diamines proved efficient irreversible amine donors with various ATA. Indeed, the amino-carbonyl by-product formed from o-xylylenediamine[15] and but-2-ene-1,4-diamine[16] are readily cyclized and subjected to further aromatization and polymerization. Interestingly, several bio-based diamine including putrescine, cadaverine and spermidine were efficiently used as smart amine donors with ATA.[17]

Besides cyclic imine formation from diamino substrates, other transamination by-product formed from polyfunctional amine donors are prone to cyclisation. Noteworthy, reactions catalyzed by glutamine-TA (GlnTA, EC 2.6.1.15) yield 2-oxoglutaramate (OGA) which was shown to exist almost exclusively (99,7%) in a cyclic hydroxylactame form[18] and some natural transaminations involving Gln as donor were reported to be displaced towards OGA formation.[18], [19]

Herein, we report the design of a new activity assay for TA using L- or D-Gln as amine donor, the implementation of this assay to identify L- and D-α-TA from biodiversity active with Gln, and application examples of these newly discovered TA for the efficient synthesis of valuable unusual amino acids. Hence, we demonstrate that Gln can truly be considered as a smart amine donor for equilibrium-shifted enzymatic transaminations.

Results and Discussion

New TA activity assay with Gln as amine donor. Assuming that Gln could be a substrate for various TA from biodiversity,[20] we firstly developed a continuous spectrophotometric assay in a 96-well microplate format in order to precisely quantify transamination activities with Gln as amine donor and various keto acid precursors of amino acid targets. As shown in Scheme 1, our assay was based on 2 auxiliary enzymes: an ω-amidase from *Arthrobacter nicotinovorans* (ωA, Uniprot ID: Q93NG1) catalyzing the hydrolysis of OGA amide into 2-oxoglutaric acid (OG)[21] and a 2-oxoglutarate reductase from *Acidaminococcus fermentans* (OGR, Uniprot ID: D2RJU7) which catalyzes the reduction of OG by NADH, thus allowing its spectrophotometric monitoring at 340 nm (ε = 6220 $M^{-1}.cm^{-1}$).[22] One NADH-based assay of OGA was already described using ωA and glutamic-dehydrogenase (GluDH).[23] However reductive amination catalyzed by GluDH requires the addition of ammonium ions which could impair TA activity. That is why OGR was preferred to GluDH for OG titration.



Scheme 1. Enzymatic assay for transaminase with Gln as amine donor.

Both auxiliary enzymes, ωA and OGR, were cloned and overexpressed in *E. coli* with addition of an His-Tag for easy purification (see table S1). Approximately 150,000 U of purified OGR were obtained from 1 L culture of the over-expressing strain. OGR proved to be highly active with a specific activity close to 1600 $U.mg^{-1}$. In the case of ωA, a

total activity of approximately 500 U was obtained from 1 L microbial culture, corresponding to a modest specific activity of 2.4 U.mg^{-1}. However, it should be pointed out that ωA activity was measured with OGR as auxiliary enzyme in non-optimal conditions, using a sample of OGA that was shown by ^{1}H NMR to be almost exclusively cyclized in aqueous solution. Considering that the acyclic form of OGA is the true substrate of ωA,[18] it is likely that the activity was underestimated in this assay and it was expected that ωA should display a much higher specific activity in the multienzymatic assay described in scheme 1 where OGA hydrolysis can compete efficiently with cyclisation. Moreover Both enzymes showed good stability and could be stored for few months without noticeable loss of activity.

In order to adjust the parameters and evaluate the performances of the new Gln-TA assay, we used as model enzyme, a D-amino acid-TA from *Enterococcus italicus DSM 15952 (*D-TA$_{Ei}$, Uniprot ID : E6LHY8*)* which was previously identified as a highly active broad spectrum TA.[20], [24] The activity of D-TA$_{Ei}$ towards D-Gln (40 mM) was first evaluated in the presence of Pyr (10 mM) as acceptor substrate by monitoring Pyr consumption using lactic dehydrogenase (LDH). In these conditions, a specific activity of 45 U.mg^{-1} was measured. D-TA$_{Ei}$ (20 mU) was then combined with various amounts of ωA and OGR in the Gln-TA assay. We could observe that 0.4 U OGR and 40 mU ωA are sufficient to observe non-limiting auxiliary reactions and these conditions were fixed for the following experiments. The apparently low needed amount of ωA confirmed the assumption that ωA specific activity measured with cyclic OGA was largely underestimated.

As shown in figure S1, the calibration curve using various amounts of D-TA$_{Ei}$ showed a broad dynamic range with a good linearity up to 20 mU/well and a calculated limit of detection of 0.3 mU/well. Finally, we considered that possible hydrolytic activity of ωA towards Gln could generate a false positive signal. Indeed, hydrolysis of Gln would give Glu which is a standard amine donor for most L-α-TA. In order to rule out this possibility, Branched-Chain-aminotransferase from *Escherichia coli* (BCAT$_{Ec}$) was used as a negative control enzyme. BCAT$_{Ec}$ activity was measured in our assay with L-Gln as donor and its natural substrate 4-methyl-2-oxopentanoic acid (MOP) as acceptor. At the same time, BCAT$_{Ec}$ activity was also measured in the same conditions with L-Glu as amine donor. Specific activities of 0.02 and 11.5 mg.mL^{-1} were measured with Gln and Glu respectively, confirming the very low activity of BCAT towards Gln and indicating that no false positive signal comes from Gln hydrolysis.

Hence, this new Gln-TA assay appeared well adapted to perform the screening of TA collections in order to highlight transamination activities with L- or D-Gln as amine donor.

Screening of a TA collection. Screening experiments were performed on a set of 22 L-TA from various classes and 2 D-TA including D-TA$_{Ei}$ (see table S1) that were identified in previous experiments for their synthetic potential with various keto acid acceptors and Glu as standard amine donor.[20] A series of 9 keto acids were used as acceptor substrates, including precursors of canonical amino acids (glyoxylic acid (GL), Pyr, MOP, hydroxypyruvic acid (HPA) and phenylpyruvic acid (PhP)) as well as unusual amino acids (2-oxobutyric acid (OB), trimethylpyruvic acid (TMP), phenylglyoxylic acid (PhG) and 2-oxo-4-phenylbutyric acid (OPB)). Recombinant *E. coli* strains overexpressing each of the 24 TA were cultured in 96-well microplates and cell-lysates were directly used in the Gln-TA assay. For each substrate, a cell lysate from host *E coli* without overexpressed TA was used as control in order to discard false positives coming from side reactions catalysed by the host-cell enzymes. The average protein content of cell lysates was 1 mg.mL^{-1} and 0.5-5 µg TA were used in each assay. In these conditions, the 2 D-TA and 5 L-TA among 22 showed activities > 0.1 U/mL of lysate with at least one keto acid substrate (table 1). The best activities in D- and L-series were measured with D-TA$_{Ei}$ and L-TA from *Megasphaera elsdenii DSM 20460* (L-TA$_{Me}$, Uniprot ID: G0VQA2) respectively. Although L-TA$_{Me}$ displayed lower activity levels than those of D-TA$_{Ei}$, a broad substrate spectrum was evidenced for both enzymes.

Table 1. Transamination activities (U.mL^{-1}) of 22 L-TA and 2 D-TA[a)] measured in cell lysates with L- or D-Gln as amine donor and various acceptor substrates [b)].

	Uniprot ID.	GL	Pyr	OB	MOP	TMP	HPA	PhG	PhP	OPB
L-TA1	G0VQA2	0,10	0,12	0,15	0,19	0,03	0,01	0,18	0,09	0,06
L-TA2	B2JE86	0,04	0,15	0,13	0,15		0,01	0,15	0,06	0,03
L-TA3	B7WY27			0,03	0,02			0,03		
L-TA4	DOMDHO	0,02	0,07	0,04	0,03		0,02		0,03	
L-TA5	G8R8R4		0,11				0,03	0,01		
L-TA6	E1VWL8	0,02	0,05		0,02		0,03	0,01	0,01	
L-TA7	C1CZ11	0,02	0,04	0,06	0,02		0,03		0,02	
L-TA8	Q1J263	0,01							0,03	
L-TA9	Q9RRM7	0,02	0,07	0,04	0,03		0,02		0,03	
L-TA10	C0GI63		0,05	0,06	0,01			0,05		0,01
L-TA11	C4LDS0			0,02	0,03			0,04		
L-TA12	Q0AV15		0,01							
L-TA13	Q0AT08									
L-TA14	Q3SH06		0,08	0,08	0,01		0,01	0,06		0,01
L-TA15	B7X3V3	0,03	0,04				0,05	0,01		
L-TA16	Q72ES5	0,03	0,06				0,05	0,01	0,02	
L-TA17	E1Q1V7	0,02					0,01	0,01	0,01	
L-TA18	A0A0H3K2P4	0,06	0,04	0,07			0,09	0,01	0,04	0,07
L-TA19	C5CIR2		0,02							
L-TA20	A1HUC4	0,09	0,02				0,11	0,01		
L-TA21	F6B3V8	0,03	0,17	0,01			0,09	0,01	0,01	
L-TA22	F6CII1	0,01	0,08				0,03		0,02	
D-TA1	P54694	0,50	0,78	0,60	0,04	0,01	0,25		0,07	0,18
D-TA2	E6LHY8	2,43	13,78	14,45	4,36	0,01	0,98	0,08	14,10	16,27

[a)] Activities of L-TA and D-TA are highlighted in blue and red respectively and are given after subtraction of the control activities measured for each substrate without overexpressed TA.[b)] Glyoxylic acid (GL), pyruvic acid (Pyr), 2-oxobutyric acid (OB), 4-methyl-2-oxopentanoic acid (MOP), trimethylpyruvic acid (TMP), hydroxypyruvic acid (HPA), phenylglyoxylic acid (PhG), phenylpyruvic acid (PhP), 4-phenyl-2-oxobutanoic acid (OPB).

Table 2. Apparent kinetic parameters of transaminations catalysed by D-TA$_{Ei}$ and L-TA$_{Me}$

TA	Substrate	K_M (mM)	k_{cat} (min^{-1})	k_{cat}/K_M (min^{-1}.mM^{-1})	Specific activity (U.mg^{-1})
D-TA$_{Ei}$	**D-Gln** [a]	9.0 ± 0.1	1904 ± 28	211 ± 5	57 ± 1
	Pyr [b]	0.9 ± 0.1	1631 ± 16	1812 ± 219	49 ± 1
	OB [b]	0.6 ± 0.1	1266 ± 14	2110 ± 375	38 ± 1
	OPB [b]	0.5 ± 0.1	836 ± 31	1672 ± 396	25 ± 1
L-TA$_{Me}$	**L-Gln** [a]	29.9 ± 1.8	39 ± 2	1.3 ± 0.1	0.9 ± 0.1
	Pyr [b]	0.3 ± 0.1	22 ± 1	73 ± 28	0.5 ± 0.1
	OB [b]	0.9 ± 0.2	21 ± 1	23 ± 6	0.5 ± 0.1
	TMP [b]	1.4 ± 0.1	5.6 ± 0.1	4.0 ± 0.4	0.12±0.01
	OPB [b]	1.1 ± 0.1	83 ± 8	75 ± 14	1.8 ± 0.2

a) variable concentrations of D- or L-Gln were used in combination with 5 mM Pyr. [b] variable concentrations of keto acid were used in combination with 40 mM D- or L-Gln.

In the case of D-TA$_{Ei}$, activities ≥ 1 U.mL^{-1} were measured with all substrates except TMP and PhG. In the case of L-TA$_{Me}$ activities were always ≥ 0.1 U.mL^{-1} except with TMP and HPA. However L-TA$_{Me}$ activity with TMP was significantly higher than the control indicating that L-TA$_{Me}$ could be used for L-*tert*-leucine (Tle) synthesis from TMP and Gln.

Kinetic parameters. In order to better characterize both enzymes and to confirm screening results, the Gln-TA assay was used with purified D-TA$_{Ei}$ and L-TA$_{Me}$, to determine kinetic parameters of transamination reactions with Pyr, KB, TMP or OPB as acceptor and L- or D-Gln as amine donor (table 2).

Enzyme kinetics clearly confirmed the greater activity of D-TA$_{Ei}$ over L-TA$_{Me}$ due to a better affinity towards the amine donor D-Gln as well as a 10 to 75 fold increased k_{cat} with the various substrates. The inactivity of D-TA$_{Ei}$ with TMP was confirmed whereas a modest activity was evidenced for L-TA$_{Me}$ with this substrate. Moreover, L-TA$_{Me}$ showed lower K_M and higher k_{cat} with all other acceptor substrates, and the best specific activity of this enzyme was measured with OPB.

In order to better characterize the substrate spectrum of both TA, activities with OPB and D/L-Gln were compared to those measured with D/L-Glu and D/L-Ala as standard amine donors. Results reported in figure 1 indicated that although D-TA$_{Ei}$ showed comparable activities with the 3 tested donors, L-TA$_{Me}$ displayed a marked preference for Glu with a ten-time activity reduction when Gln was used as amine donor. Nevertheless, L-TA$_{Me}$ was considered as a suitable enzyme to demonstrate the relevance of the equilibrium shift strategy using Gln as amine donor.

Synthetic experiments. As a first model reaction, the transamination reaction between Pyr and L-Gln was studied using L-TA$_{Me}$ as catalyst. In a first analytical experiment, Pyr (50 mM) was reacted with L-Gln at various concentrations (0-60 mM).

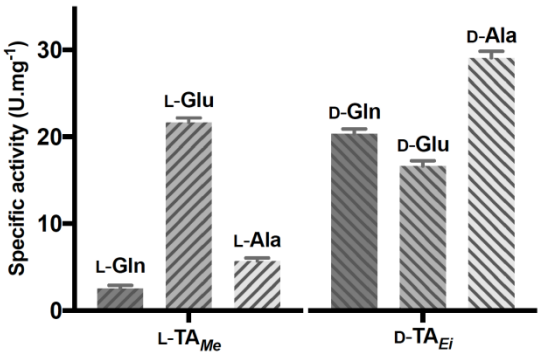


Figure 1. Activities of D-TA$_{Ei}$ and L-TA$_{Me}$ with OPB (1 mM) and various amine donors (40 mM): OGR or LDH were used as auxiliary enzymes for the reduction by NADH of OG or Pyr formed from D/L-Glu and D/L-Ala respectively.

Residual Pyr was titrated over time with LDH and NADH until equilibration (stabilization of Pyr concentration). In order to highlight the interest of Gln as amine donor, the same experiment was run using L-Glu as amine donor. Final Pyr concentrations measured at various initial donor/Pyr ratios are reported in Figure 2. As expected, we could observe a linear correlation between residual [Pyr] at equilibrium and the initial donor/Pyr ratio indicating an apparently complete reaction and therefore a very efficient equilibrium shift afforded by OGA cyclisation. Considering that the conversion obtained with one molar equivalent of Gln was above 98%, indicated an equilibrium constant over 2000 for the transamination reaction between Pyr and Gln. Conversely, when using a stoichiometric amount of Glu as amine donor, the conversion was less than 60%, thus indicating an equilibrium constant lower than 2.25. A larger-scale reaction between Pyr (50 mM, 0.7 mmol) and Gln (60 mM) was then run using L-TA$_{Me}$ (9.3 µM) as catalyst. In those conditions,

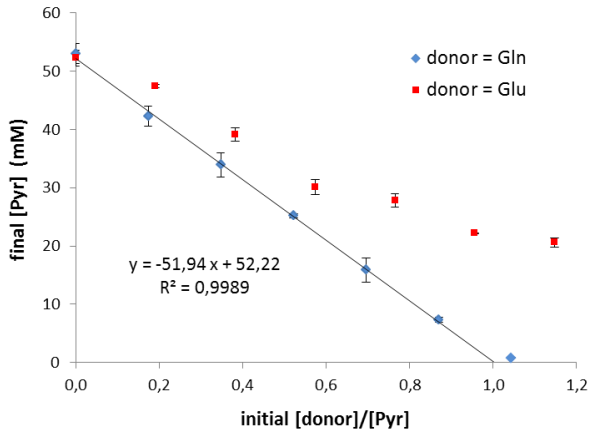
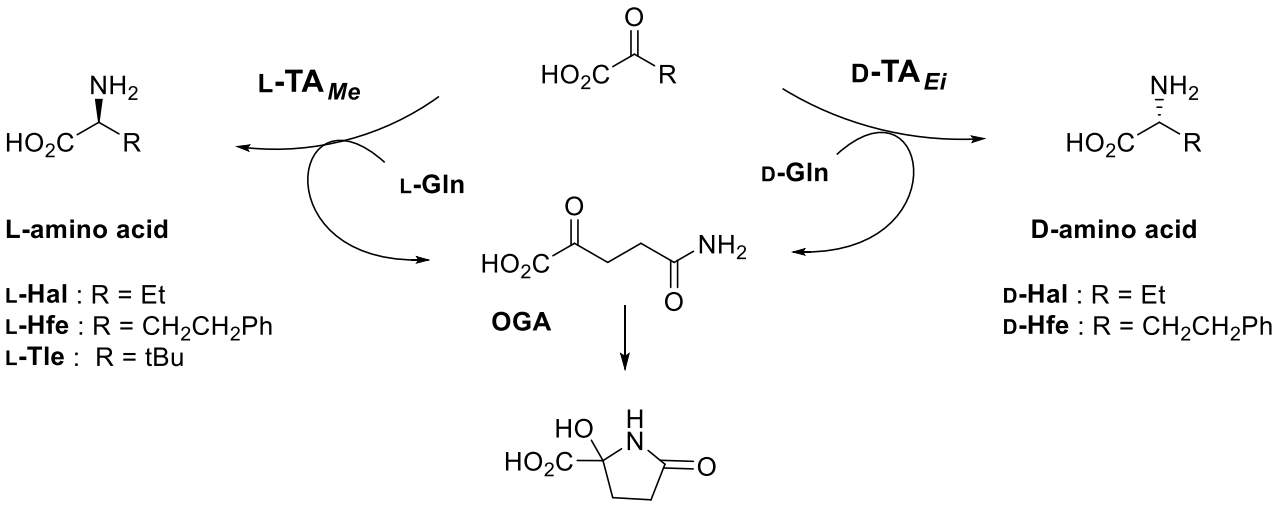


Figure 2. Final concentrations of Pyr measured in transaminations with various initial [donor]/[Pyr] ratios.

the conversion rate reached 95% in less than 1.5 h and Pyr titration after 3 h indicated a completely displaced reaction (> 99% conversion). Amino acids were removed from the reaction mixture by selective adsorption on a sulfonic acid containing ion exchange resin and the lithium salt of OGA was quantitatively isolated after neutralization with lithium hydroxide and lyophilisation. NMR analyses showed that OGA was the only keto acid present in the reaction mixture and confirmed that it exists almost exclusively in its cyclic form in aqueous solution (see SI). It should be pointed out that this transamination reaction between Pyr and Gln thus constitutes a highly efficient method to prepare OGA, which is an important biomarker of hyperammonemia.[25]

In light of these first encouraging results, L-TA$_{Me}$ and D-TA$_{Ei}$ were evaluated as catalysts for the synthesis of valuable unusual L- or D-amino acids using L- or D-Gln as amine donor (scheme 2). In accordance with the substrate spectra evaluated through screening and kinetic experiments, L-TA$_{Me}$ was assayed for the synthesis of L-homo-alanine (Hal), L-*tert*-leucine (Tle) and L-homophenylalanine (Hfe). These three amino acids are included in the peptidomimetic structures of widely used drugs[1] and their stereoselective synthesis has been the subject of many studies.[2], [26] Furthermore, the potential of D-TA$_{Ei}$ was assessed through the synthesis of D-Hal and D-Hfe which also constitute important building blocks for biologically active compounds.[27]

Synthesis experiments were run on 0.1 mmol scale and 100 mM Gln and 120 mM OB or TMP were first chosen as standard substrate concentrations. A slight excess of acceptor was used in order to achieve a complete conversion of Gln allowing the isolation of pure amino acid products by selective adsorption on a cation exchange resin. Enzymes specific activities were measured in these conditions in order to address possible inhibition at high substrate concentrations. In the case of D-TA$_{Ei}$, the results were very close from those reported in table 1 (36 and 28 U.mg^{-1} for OB and OPB respectively). Specific activities of L-TA$_{Me}$ with 120 mM OB or TMP and 100 mM L-Gln (1,8 and 0.7 U.mg^{-1} respectively) were found higher than those measured previously at 40 mM L-Gln (table 1). These results were in accordance with the high K_M value determined for L-Gln and L-TA$_{Me}$ (table 1) and indicated that no significant substrate inhibition was observed for L-TA$_{Me}$ with these 2 substrates. In the case of the lithium salt of OPB, a maximum solubility of 14 mM was determined in water at pH 7.6. Consequently, reactions were run in heterogeneous conditions with a suspension of OPB (18.4 g.L^{-1}, theoretically 100 mM). As Hfe is even less soluble in water (maximum solubility < 3 mM), it was simply isolated by filtration at the end of the reaction. For that reason, a slight excess of Gln (120 mM) was used in that case to ensure a complete conversion of OPB. Syntheses results are summarized in table 3. Amino acids of L- or D-series were isolated with excellent enantiopurity, confirming again that TA are highly enantioselective catalysts. All reactions showed conversions over 90%, clearly demonstrating that using Gln as amine donor is an effective and

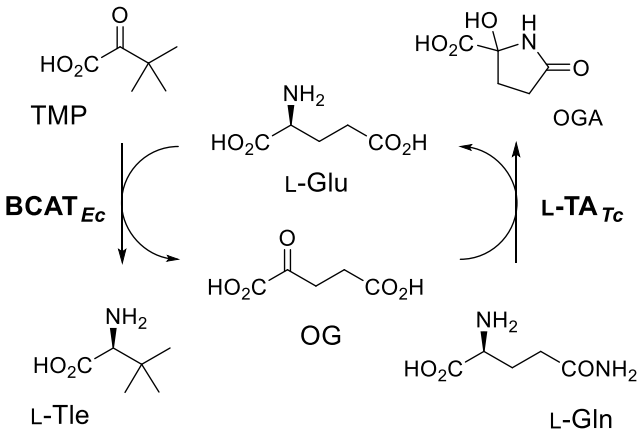


Scheme 2. Synthesis of unsual L- and D- amino acids using L- or D-Gln as amine donor.

Table 3. Synthesis of unsual amino acids catalysed by L-TA$_{Me}$ and D-TA$_{Ei}$ with L- or D-Gln as amine donor

TA (µM)	**Acceptor** (mM)	**Donor** (mM)	**Time** (h)	**Product**	**Conversion**[a] (%)	**ee**[b] (%)
L-TA$_{Me}$ (65)	OB (120)	L-Gln (100)	24	L-Hal	98	> 99
L-TA$_{Me}$ (130)	TMP (120)	L-Gln (100)	48	L-Tle	92	> 99
L-TA$_{Me}$ (90)	OPB (100)	L-Gln (120)	24	L-Hfe	93	> 99
D-TA$_{Ei}$ (8.4)	OB (120)	D-Gln (100)	7	D-Hal	99	> 99
D-TA$_{Ei}$ (8.4)	OPB (100)	D-Gln (120)	24	D-Hfe	96	> 99

[a] Conversions of the limiting substrate were calculated by NMR analysis of reaction media and were always inferior or equal to yields calculated from isolated products. [b] Enantiomeric excess determined by HPLC.



Scheme 3. Synthesis of L-Tle by coupling BCAT$_{Ec}$ with L-TA$_{Tc}$ using Gln as smart amine donor

general method to shift the equilibrium of transamination reactions.

It might be argued however that Gln is maybe not commonly accepted as a good substrate by L-α-TA and that finding one enzyme showing a good activity for both Gln and the keto acid precursor of a valuable target might be a cumbersome task. Nevertheless, a Gln-TA could also be used as an auxiliary enzyme to shift the equilibrium of another transamination reaction catalyzed by an α-TA or an Amine-TA devoid of activity towards Gln. In this bienzymatic system, Ala or Glu could be used as standard primary amine donor ensuring the coupling between both transamination reactions. In order to illustrate this approach, we designed the new synthetic process of L-Tle described in scheme 3, by coupling BCAT$_{Ec}$ and a previously assayed serine-glyoxylate-TA from *Thermosinus carboxydivorans Nor1* (L-TA$_{Tc}$, Uniprot ID: A1HUC4). BCAT$_{Ec}$ was previously used for Tle synthesis[14], [28] whereas thermostable L-TA$_{Tc}$ was recently combined with a transketolase for *in situ* production of HPA from L-Serine.[29] This TA couple was chosen because although neither of the two TA showed any activity with TMP and Gln (< 0.02 U.mg^{-1}), BCAT$_{Ec}$ proved active with TMP and Glu (1.1 U.mg^{-1}) whereas L-TA$_{Tc}$ showed significant activity with Gln or Glu using Pyr as acceptor (0.6 or 1.1 U.mg^{-1} respectively). The process of scheme 3 was implemented on 0.1 mmol scale using TMP (110 mM), Gln (100 mM), Glu (10 mM), and 100 µM of each TA. Under these conditions, a conversion of 94% was reached in 48 h, thus demonstrating the applicability of this Gln-TA coupled approach.

Conclusion

In summary, we have developed an efficient spectrophotometric assay, which is suitable not only for screening enzyme collections but also for kinetic studies of TA which are active with L- or D-Gln as amine donor. The screening of a small collection of 24 TA highlighted several L- or D-TA showing significant activity with L- or D-Gln. The 2 most active enzymes in L- and D-series were used for high yielding and enantioselective synthesis of three valuable unusual amino acids, thus demonstrating that Gln, a readily available and bio-based substrate, can be used as a smart amine donor in order to shift the equilibrium of transamination reactions. Further large scale screening experiments will undoubtedly allow the discovery of highly active and robust new Gln-TA for use as catalyst or auxiliary enzyme in large scale and highly enantioselective syntheses of a variety of chiral amines.

Experimental Section

General procedure for amino acid synthesis using L-TA$_{Me}$ or D-TA$_{Ei}$. Reactions were carried out in Eppendorf tubes (1,5 mL). To a 1 mL aqueous solution containing L- or D-Gln (0.1 or 0.12 mmol), OPB (0.1 mmol), OB (0.12 mmol) or TMP (0.12 mmol) and PLP (0.1 µmol) at pH 8 was added L-TA$_{Me}$ (6.5-13 nmol) or D-TA$_{Ei}$ (0.84 nmol): a suspension of the TA in 3 M $(NH_4)_2SO_4$ was centrifuged (14000 rpm, 5 min.), the supernatant was discarded and the pellet diluted with the solution of substrates and cofactor. The reaction mixture was orbitally stirred at room temperature and controlled by thin layer chromatography. After 7 to 48 h, L- or

D-Hfe was isolated by filtration on a 0.22 µm membrane. The white solid was washed with H_2O (4x0.5 mL) and dried under vacuum (0.5 mm Hg) for 24 h at 40 °C. In the case of Hal and Tle, the reaction mixture was poured on a small column of dowex® 50WX8 (H^+ form, 1 mL). The column was washed with H_2O (10 mL) before elution with 1 M NH_3. Ninhydrin positive fractions were pooled and concentrated under reduced pressure. Amino acids were isolated as white solids. Conversions, chemical and enantiomeric purity were assessed by NMR and HPLC analyses (See SI).

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Egon Heuson, Franck Charmantray, Jean-Louis Petit, Véronique de Berardinis, and Thierry Gefflaut [a]*