Tetrahedron Letters, Vol. 35, No. 1, pp. 29-32, 1994
Printed in Great Britain

0040-4039/94 $6.00 + .00
Pergamon Press Ltd

Enantiomeric Conversion of Racemic Amino Acid Mixtures via an Oxidase-Aminotransferase Coupled System

Sapan A. Shah, Peter H. Schafer, Paul A. Recchia, Kevin J. Polach and David M. LeMaster*

Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois 60208 USA

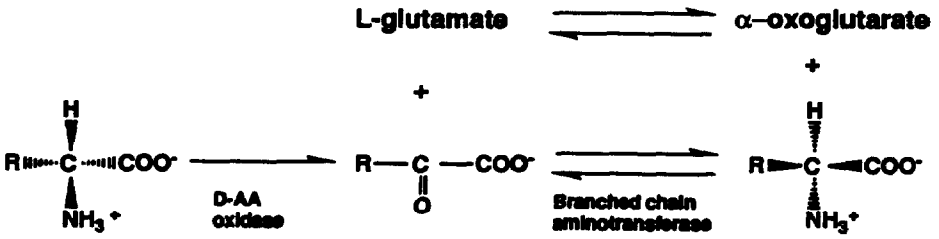
Abstract: D-amino acid oxidase, branched-chain amino acid aminotransferase and excess L-glutamate are used to convert racemic mixtures to the L-form enantiomer in high chemical and optical yields for a number of both common and non-biological amino acids.

Stereoselective procedures for the synthesis of amino acids have been developed which are suitable for the chiral introduction of each of the four groups attached to the alpha carbon.[1] For many applications these chiral syntheses offer a clear advantage over racemic procedures only when sufficiently high enantiomeric purity is obtained so as to obviate the need for an often laborious resolution procedure. On the other hand, racemic syntheses generally utilize simpler and more readily available starting materials. This is particularly relevant for isotopic labeling syntheses since the initial form of the isotope often strongly circumscribes the practical synthetic pathways. Development of a straightforward means to convert the undesired (usually D-form) to the desired (usually L-form) enantiomer would serve to overcome the dominant limitation of racemic amino acid synthetic procedures. We report a one-pot enantiomeric conversion system in which the intermediate α-oxo acid, generated via D-amino acid oxidase, is converted in situ to the corresponding L-form amino acid.

Several studies have generated L-amino acids from the corresponding α-oxo acids either directly or via a coupled system using NAD(P)H dependent glutamate,[2] malate[3] or leucine + formate[4] dehydrogenases. These redox-coupled systems exhibit a limited substrate range. More importantly they are restricted by the chemical instability of NAD(P)H, particularly in the presence of oxygen. With the exception of a 100 μmol enantiomeric conversion of D,L-methionine using the leucine dehydrogenase,[4] these redox enzyme procedures have made use of the independently generated α-oxo acids.

The oxygen sensitivity of redox enzyme catalyzed enantiomeric conversion has been circumvented for cyclic imino acids[5] by making use of inorganic reductants. D-proline and D-pipecolic acid are excellent substrates for the porcine D-amino acid oxidase.[6] Since sodium borohydride does not significantly inhibit this enzyme,[7] it can be used to reduce the protonated iminium group formed so as to regenerate the amino acid, 50% of the time as the desired L-form. Unfortunately, the decreased hydrolytic stability of the intermediate imine renders this approach far less fruitful for acyclic amino acids.[7]

We have examined the comparatively oxygen insensitive E. coli branched-chain amino acid aminotransferase as a means of converting the intermediate α-oxo acids to the L-form amino acids as illustrated in Scheme 1. Genetic studies[8] indicated a rather broad specificity for the E. coli branched-chain amino acid aminotransferase which prompted our cloning and overexpression of the corresponding gene.[9] The initial biochemical studies[9,10] suggest the specificity of the aminotransferase is nearly as broad as that of the widely studied oxidase.[6]



Scheme 1

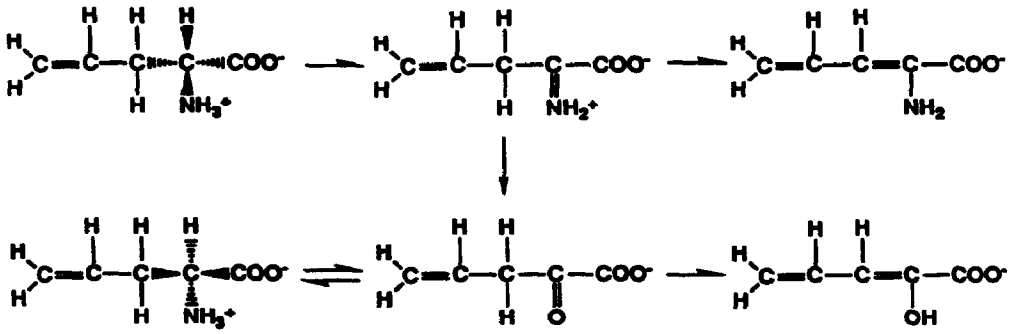
Having circumvented the dehydrogenase-driven reaction, an alternate means is required to shift the equilibrium toward the desired L-amino acid. We have found quite useful the simple expedient of adding an excess of the physiological amine donor L-glutamate. Monosodium glutamate is soluble to above 0.5 M. We observed negligible inhibition of either the oxidase or transaminase reaction under these conditions. Cost is of limited concern as due to its use in the food additive industry, L-glutamate is produced at a level of $5x10^5$ tons/year at a cost of around $6/kg.[11] The only practical problem is the need to remove the 10-fold excess of L-glutamate standardly used in these reactions. However, the negative charge on the sidechain makes the separation of glutamate from the neutral amino acids straightforward via ion exchange chromatography.

Table 1. Oxidase-Aminotransferase Enantiomeric Conversion Yields

D,L Amino Acid	Scale (mmoles)	[BCAT] (U/ml)	[oxidase] (U/ml)	yield (gram-%)	ee
Valine	10	0.025	0.1	1.05 (90%)	99.1
Leucine	10	0.025	0.2	1.14 (87%)	99.6
Isoleucine	10	0.05	0.2	1.14 (87%)	96.7
Methionine (72hr)	10	0.025	0.1	1.07 (72%)	99.5
Methionine (24hr)	10	0.075	0.3	1.34 (90%)	>99.8
Phenylalanine	10	0.125	0.2	1.57 (96%)	98.7
2-Aminobutyrate	5	0.25	0.2	0.47 (91%)	99.7
Allylglycine	5	0.25	0.2	0.35 (61%)	98.5

The first six entries of Table 1[12] indicate the results for several standard amino acids. Enantiomeric excesses of approximately 99% were obtained as determined by HPLC analysis of the 2,4-dinitrophenyl-5-L-alanine amide derivatives (ie. Marfey's reagent).[13] The exception is "D,L isoleucine" which is in fact a mixture of all four isomers at the α and β positions. The reduced enantiomeric purity of this product presumably reflects the lower activity of the D-amino acid oxidase on D-alloisoleucine as compared to D-isoleucine.[6] In all cases the residual D-form amino acid is a reflection of the amount of D-amino acid oxidase used. The amount of oxidase can be increased when higher enantiomeric purity is required. With the exception of methionine, the recovered yields for the three day protocol are approximately 90%. In this case it was suspected that instability of the intermediate α-oxo acid might have contributed to the reduced yield for methionine. A one day methionine experiment was conducted using a 3-fold increase in the amount of oxidase and aminotransferase and a 90% yield was obtained.

Further experiments were conducted to examine the utility of this enantiomeric conversion system for the production of nonstandard amino acids since efficient enzymatic catalysis is often limited to the natural biological substrates. For both 2-aminobutyric acid and 2-amino-4-pentenoic acid (allylglycine) there is approximately a hundred-fold price differential between the racemic and the pure L-form. 2-Amino-4-pentenoic acid (allylglycine) offers a particularly interesting case. Marcotte and Walsh[14] have shown that the D-amino acid oxidase product 2-iminium-4-pentenoate rapidly tautomerizes to the potent noncovalent inhibitor 2-amino-2,4-pentadienoate as illustrated in Scheme 2. Even if the initial iminium intermediate hydrolyzes to the 2-oxo form, an analogous tautomerization yields the strongly inhibitory 2-hydroxy-2,4-pentadienoate. By directly coupling this D-amino acid oxidase reaction to transamination, conversion to the desired L-amino acid successfully competes for the intermediate 2-oxo acid. As a result the oxidase enzyme is more efficiently utilized. Furthermore, since less 2-amino-2,4-pentadienoate and 2-hydroxy-2,4-pentadienoate is formed, less of the sample undergoes the additional irreversible rearrangement to the fully conjugated molecules.[14]



Scheme 2

The case of allylglycine illustrates the general advantage of directly coupling the oxidase reaction with transamination in order to minimize the accumulation of the intermediate α-oxo acids. Although the degree of inhibition is strongly dependent on the α-oxo acid in question (fortunately α-oxoglutarate is a very poor inhibitor[15]), a K_I of 1-5 mM is observed for a number of the common α-oxoacids.[16] The K_M values for the amino acids used in this study similarly lie near 1 mM.[16]

This coupled oxidase-aminotransferase system is quite robust in terms of consistently high product yields. This enantiomeric conversion system is amenable to substantially larger scale reactions than have been reported here. Porcine D-amino acid oxidase is already widely used commercially and the amount of E. coli branched-chain amino acid aminotransferase used in the ten millimole valine reaction presents less than 0.05% of that obtained from a standard 10 liter fermenter culture. Initial studies indicate that the substrate range can be significantly broadened. It should be noted that only one equivalent of L-glutamate is consumed for each equivalent of D-amino acid converted. The chromatographic isolation procedure allows for direct recycling of the remaining L-glutamate as warranted.

Acknowledgment: This work was supported by Project Grant GM-38779 from the National Institutes of General Medical Sciences, the Presidential Young Investigator Award Grant DMB-8957336 from the National Science Foundation.

References and Notes

1. Duhamel, L.; Duhamel, P.; Fouquay, S.; Eddine, J. J.; Peschard, O.; Plaquevent, J. C.; Ravard, A.; Solliard, R.; Valnot, J. Y.; Vincens, H. *Tetrahedron* **1988**, *44*, 5495-5506.
2. Greenaway, W.; Whatley, F. R. *FEBS Lett.* **1977**, *75*, 41-43.
3. Baldwin, J. E.; Dyer, R. L.; Ng, S. C.; Pratt, A. J.; Russell, M. A. *Tetrahedron Lett.* **1987**, *28*, 3745-3746.
4. Nakajima, N.; Esaki, N.; Soda, K. *J. Chem. Soc. Chem. Commun.* **1990**, 947-948.
5. Chang, Y. F.; Charles, A. K.; Tilkin, R. B. *Anal. Bioch.* **1982**, *125*, 376-385.
6. Greenstein, J. P.; Birnbaum, S. M.; Otey, M. C. *J. Biol. Chem.* **1953**, *204*, 307-321.
7. Hafner, E. W.; Wellner, D. *Proc. Natl. Acad. Sci. USA* **1971**, *68*, 987-991.
8. Gelfand, D. H.; Steinberg, R. A. *J. Bacteriol.* **1977**, *130*, 429-440.
9. Chanatry, J. A.; Schafer, P. H.; Kim, M. S.; LeMaster, D. M. *Analyt. Bioch.* **1993**, in press.
10. Inoue, K.; Kuramitsu, S.; Aki, K.; Watanabe, Y.; Takagi, T.; Nishigai, M.; Ikai, A.; Kagamiyama, H. *J. Biochem.* **1988**, *104*, 777-784.
11. Martens, J. *Top. Curr. Chem.* **1984**, *125*, 165-240.
12. For example:
Ten mmol of D,L-valine and 100 mmol of monosodium glutamate were dissolved in 200 ml of 100 mM Tris HCl pH 8.5 containing 1mM EDTA. To this solution were added in order: 200 mg bovine serum albumin, 10 mg pyridoxal 5'-phosphate, 3.0 mg of catalase, 20 U of D-amino acid oxidase and 5 U of branched-chain amino acid aminotransferase. The flask was incubated in the dark at 37^{0}C with agitation for 3 days. The diluted sample was passed onto a Dowex 50 X-8 column, and the valine and glutamate were displaced with 150 mM pyridine. After rotary evaporation the sample was resuspended in water and loaded onto a Dowex 1 column in acetate form. The L-valine solution passed directly through the column and was concentrated to a crystalline powder.
13. Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591-596.
14. Marcotte, P.; Walsh, C. *Biochemistry* **1978**, *17*, 5620-5626.
15. Hamilton, G. A.; Buckthal, D. J. *J. Bioorg. Chem.* **1982**, *11*, 350-370.
16. Dixon, M.; Kleppe, K. *Biochim. Biophys. Acta* **1965**, *96*, 368-382.

(*Received in USA* 12 *August* 1993; *revised* 14 *October* 1993; *accepted* 25 *October* 1993)