Enzymatic Conversion of Racemic Methionine to the L-Enantiomer

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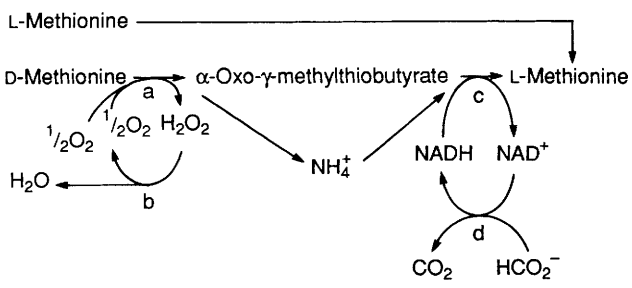
We have developed an enzymatic method for conversion of racemic methionine to the L-enantiomer with a yield of >95% and >99% enantiomeric excess; the method has been applied to the conversion of DL-alanine and DL-leucine to the L-enantiomers.

Racemic amino acids may be resolved to their L- and D-enantiomers by enzymatic or chemical procedures.[1] No methods, however, are available for a total conversion of the racemate to only one enantiomer. L-Methionine is used widely as a feed additive, a component of infusion, and a starting material for pharmaceutical synthesis, but microbial methods have not been developed for L-methionine production. We here describe a simple method for the production of L-methionine from the racemate by means of D-amino acid oxidase (EC 1.4.3.3), catalase (EC 1.11.1.6), leucine dehydrogenase (EC 1.4.1.9), and formate dehydrogenase (EC 1.2.1.2). D-Methionine is fully converted to the L-enantiomer *in situ* through an achiral intermediate, α-oxo-γ-methylthiobutyrate, in an enzymatic system containing oxygen, NAD^+, and ammonium formate (Scheme 1).



Scheme 1. Reaction scheme for the enzymatic conversion of DL-methionine to the L-enantiomer: a, D-amino acid oxidase; b, catalase; c, leucine dehydrogenase; d, formate dehydrogenase.

Leucine dehydrogenase catalyses the reversible deamination of various aliphatic L-amino acids including methionine to their α-oxo analogues in the presence of NAD^+.[2] The reaction is favourable for reductive amination, which is accelerated by combination with the formate dehydrogenase reaction. Formate dehydrogenase catalyses the irreversible oxidation of formate to CO_2 with concomitant reduction of NAD^+ to NADH.[3] α-Oxo acids are spontaneously decarboxylated by oxidation with H_2O_2. Thus catalase is added to the reaction mixture containing the purified enzymes to decompose H_2O_2,

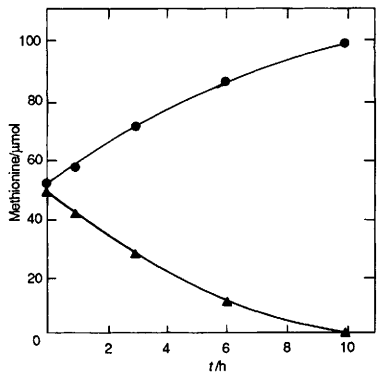


Figure 1. Enzymatic conversion of racemic methionine to the L-enantiomer. The enzymatic coupled reaction was performed under the conditions described in the text; (●): L-methionine, (▲): D-methionine.

but is not necessary in the crude enzyme system, because catalase occurs ubiquitously in organisms.

We found that D-methionine is converted to the L-enantiomer most efficiently under the following conditions. The reaction mixture contained DL-methionine (100 μmol), NADH (1 μmol), ammonium chloride (25 μmol), sodium formate (500 μmol), Tris–HCl buffer (pH 8.5; 100 μmol), D-amino acid oxidase from Sigma (5 units), catalase from Sigma (2 units), leucine dehydrogenase from *Clostridium thermoaceticum* AN 28-4 (10 units),[2] and formate dehydrogenase from Boehringer (2 units) in a final volume of 1 ml (Figure 1). The optimum temperature for the reaction was about 37 °C, and the optimum pH was between 8.0 and 8.5. After incubation for 10 h, HCl was added to the reaction mixture to a final concentration of 1.0 M. The solution was applied to a Dowex 50(H^+) column (1 × 10 cm), and L-methionine was eluted with 1 M NH_4OH. The fractions containing L-methionine were pooled and concentrated to a small volume, followed by evaporation to dryness under reduced pressure. The residue was dissolved in a small volume of hot 80% ethanol, and L-methionine was crystallized at 4 °C.

Enantioselective HPLC with a Daicel Crown pack column (0.4 i.d. × 50 cm) showed that D-methionine was completely converted to the L-enantiomer (>99% enantiomeric excess, e.e.). The yield of L-methionine was >95% based on DL-methionine used. No D-enantiomer of methionine was detected by enantioselective ligand exchange chromatography or by the D-amino acid oxidase method.[4] The optical rotation value at 436 nm of the product isolated coincided with that of the authentic L-methionine. This is the first example of *in situ* conversion of a racemate completely to one of its enantiomers, and in principle is based on the conversion of D-amino acids to the L-counterparts.

We also converted 0.1 M DL-alanine to the L-enantiomer (>95% yield; >99% e.e. in 10 h) and 0.1 M DL-leucine to the L-enantiomer (95% yield; 99% e.e. in 2 h) under similar conditions.

Both D-amino acid oxidase[5] and leucine dehydrogenase[2] show strict enantioselectivity, but low substrate specificity. Therefore, this procedure should be applicable to production of L-selenomethionine, L-cysteine, L-valine, and other aliphatic L-amino acids that are substrates of leucine dehydrogenase in the same manner. The enzymes used are commercially and cheaply available, and stable.

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