Stereochemistry of the Leucine 2,3-Aminomutase from Tissue Cultures of *Andrographis paniculata*

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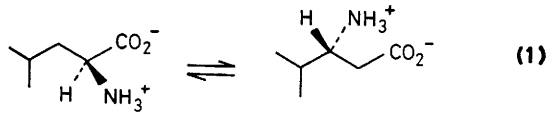
Summary A leucine 2,3-aminomutase from *Andrographis* tissue cultures mediates the equilibrium between (2*S*)-α- and (3*R*)-β-leucine; the activity does not apparently depend on coenzyme B_{12}.

SIX α-amino-acid mutases have been reported. Three are coenzyme B_{12}-dependent and effect the 1,2-shift of a terminal amino-group: (*S*)-β-lysine-5,6-aminomutase (E.C. 5.4.3.3),[1] (*R*)-α-lysine-5,6-aminomutase (E.C. 5.4.3.4),[2] and

(*R*)-ornithine-4,5-aminomutase (E.C. 5.4.3aa).[3] Of the three enzymes that effect α- to β-amino-acid conversions, (*S*)-α-lysine-2,3-aminomutase (E.C. 5.4.3.2)[4] requires pyridoxal phosphate and *S*-adenosylmethionine, (*S*)-tyrosine-2,3-aminomutase[5] requires ATP but not pyridoxal phosphate, while leucine-2,3-aminomutase, as isolated from a variety of sources by Poston, has been reported[6,7] to depend on coenzyme B_{12} for its activity. Stereochemical studies must play an important part in attempts to understand these functionally related but mechanistically diverse enzymes. So far only the (*S*)-β-leucine-[8,9] and (*S*)-tyrosine-[10] mutases have been subjected to stereochemical scrutiny. We now report on the stereochemistry of the leucine-2,3-aminomutase which we have obtained from *Andrographis* callus cultures. We show that in the equilibrium (1), mediated by this enzyme, (2*S*)-α- and (3*R*)-β-leucines are the metabolically active substrates.



(1)

The remaining stereochemical questions, whether migration of $-NH_2$ and –H occurs with retention or inversion of configuration at the two termini, is under study and will be reported elsewhere.

(*R*)- and (*S*)-α-Leucines were obtained commercially (Aldrich Chemical Co. Ltd.); (3*RS*)-β-leucine was synthesised by addition of ammonia to 4-methylpent-2-enoic acid,[11] and (3*R*)-β-leucine by Arndt–Eistert homologation of (2*S*)-valine.[12] The methyl ester camphanamides of the four amino-acids [dry HCl–MeOH), 40 °C, then camphanyl chloride (Fluka AG)–pyridine, 80 °C] were completely separable by g.l.c. on (a) 1% OV210, 7 ft, 170 °C [retention indices (r.i.) (2*R*)-α 2545, (2*S*)-α 2580, (3*R*)-β 2610, and (3*S*)-β 2630] and (b) 1% QF1, 9 ft, 200 °C [r.i. (2*R*)-α 2785, (2*S*)-α 2830, (3*S*)-β 2865, and (3*R*)-β 2890].

(2*S*)-[U-^{14}C]α-Leucine (10 μCi, 20 μmol; Radiochemical Centre, Amersham) was incubated with the 75,000 g supernatant from *Andrographis* callus cultures[13] (1 ml, 2·5 mg protein[14]) anaerobically in the dark for 16 h. Recovery of the amino-acids by chromatography over Dowex 50W-X8 (acid form) and radio-g.l.c.[13] (Pye 104-Panax system) of the derived methyl ester camphanamides on 1% OV 210 and 1% QF1 showed radioactivity only in the (2*S*)-α- and (3*R*)-β-leucines (α to β conversion 0·4%). In a complementary experiment (3*R*)-[1,3,4,5,5′-$^{14}C_5$]β-leucine (30 μmol, 0·004 μCi/μmol), prepared from (2*S*)-[U-^{14}C]valine (50 μCi + 4·3 mmol 'cold' valine; 11·6 μCi/mmol) was converted into α-leucine (β to α conversion 3%). Derivatisation as above, separation by preparative t.l.c. (Merck Kieselgel 60 F_{254}, 0·25 mm; hexane–ethyl acetate 1 : 1, R_f α-leucine 0·44, β-leucine 0·28), and radio-g.l.c. again showed radioactivity only in the (3*R*)-β- and (2*S*)-α-leucine derivatives. In blank experiments with denatured enzyme (20% $HClO_4$, 0·1 ml per ml incubate) there was no transfer of label between α- and β-leucines. These experiments demonstrate that (2*S*)-α- and (3*R*)-β-leucines are the metabolically active substrates, provided there is no undetected interference from α-leucine racemase in the cell-free preparation used. This assumption appears to be justified by the absence of labelled (2*R*)-α-leucine at the conclusion of both the above experiments. It was further supported by experiments in which there was no change in the $^3H/^{14}C$ ratio when (2*S*)-[2-$^3H_1$]- + (2*S*)-[U-^{14}C]- and (2*R*)-[2-$^3H_1$]- + (2*S*)-[U-^{14}C]-α-leucines were incubated with the cell-free system. Thus, (2*S*)-[2-$^3H_1$]- and (2*R*)-[2-$^3H_1$]-α-leucines were prepared by exchange[15] of (*RS*)-acetyl-leucine (150 mg) with tritiated water (50 μl, 250 mCi; Radiochemical Centre) (67% yield, specific activity 74·1 mCi/mmol) and resolution after dilution with unlabelled (*RS*)-acetyl-leucine (final specific activity 8·79 mCi/mmol), using hog renal acylase[16] (Sigma, London; 2600 units/mg). The specific activities of (2*S*)- (82% yield) and (2*R*)- (60% yield) α-leucines were (after crystallisation to constant activity and estimation as the derived methyl ester trifluoroacetamides) 3·15 mCi/mmol. (2*S*)-[2-$^3H_1$]- and (2*R*)-[2-$^3H_1$]-α-Leucines were each mixed with (2*S*)-[U-^{14}C]-α-leucine to give, respectively, mixtures with $^3H/^{14}C$ 8·27; 373 mCi ^{14}C/mol and 9·12; 337 mCi ^{14}C/mol. After incubation with the *Andrographis* enzymes as above, the recovered (2*S*)- and (2*R*)-α-leucines (dilution with unlabelled material, crystallisation, and conversion into methyl ester trifluoroacetamides) had $^3H/^{14}C$ 8·34; 33·2 mCi ^{14}C/mol and 9·00; 30·3 mCi ^{14}C/mol, respectively. Re-isolation of (2*S*)- and (2*R*)-α-leucines with unchanged $^3H/^{14}C$ ratios suggests,[17] even allowing for a substantial tritium-isotope effect, that α-amino-acid racemase or transaminase activity in the *Andrographis* cell-free system must be moderate.

The cell-free preparation from *Andrographis* cultures does not manifest any of the activity associated with α-[18] or β-leucine[6] catabolism in micro-organisms. Thus, the total recoverable radioactivity (*ca.* 80%) from incubation with labelled (3*R*)-β-leucine was in both β- and α-leucines. There was no detectable (radio-g.l.c.) activity corresponding to the following acids: α-oxoisocaproic, isovaleric, acetoacetic, and isobutyric.

We have failed in our initial experiments with the *Andrographis* enzyme to secure convincing evidence for B_{12}-coenzyme dependence. (3*R*)-[4,5-$^3H_2$]-β-Leucine (20 μmol, 0·26 μCi/μmol) from (2*S*)-[3,4-$^3H_2$]valine (1·92 mmol, 531 μCi/mmol; Radiochemical Centre) was incubated in five separated experiments with the 75,000 g supernatant, containing in 1 ml (a): pyridoxal phosphate (0·12 mg, Sigma, London), coenzyme A (0·38 mg, Boehringer, London), NAD (0·34 mg, Boehringer), and FAD (0·41 mg, Boehringer); (b): as (a) + *S*-adenosylmethionine (0·22 mg, Sigma); (c): as (a) + coenzyme B_{12} (6·3 mg, Sigma); (d): as (a) + intrinsic factor X10 (1 mg, ICN Pharmaceuticals, Cleveland, Ohio); (e): as (a) + 20% $HClO_4$ before addition of β-leucine. Radio-g.l.c. of the methyl ester trifluoroacetamides [1% OV17, 6 ft, 80 °C; r.i. (2*S*)-α 1234 and (3*R*)-β 1316] showed between 0·3 and 0·5% β- into α-leucine conversion, except in (e), and no detectable differences in (c) or (d). We found that denaturation of the enzyme by acid or heat produced small *and variable* amounts of α-leucine by hydrolysis, as was readily apparent in blank experiments from g.l.c. of the methyl ester trifluoroacetamides. The low levels of β- into α-leucine conversion by the mutase therefore made it necessary to monitor such

conversion, and in particular the possible effects of co-enzyme B_{12} and intrinsic factor on it, by radio-g.l.c. analysis, using labelled substrates.

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