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Leucine 2,3-Aminomutase: A Cobalamin-Dependent Enzyme Present in Bean Seedlings

Abstract. *Leucine 2,3-aminomutase has been demonstrated in extracts of bean seedlings. The activity of this enzyme is stimulated by coenzyme B_{12} and is inhibited by intrinsic factor. The inhibition is removed by the addition of coenzyme B_{12}. This evidence is consistent with the presence of a cobalamin-dependent enzyme in higher plants.*

The catabolism of leucine has long been recognized as proceeding by way of transamination with the subsequent decarboxylation of the resulting α-ketoisocaproic acid. A second catabolic pathway has recently been shown to function in microorganisms (*1*). In this pathway leucine is converted to β-leucine (3-amino-4-methylpentanoic acid) by leucine 2,3-aminomutase (E.C. 5.4.3.–), an enzyme that requires activation by coenzyme B_{12} [adenosylcob(III)alamin]; the β-leucine is deaminated, and the resulting β-ketoisocaproic acid is cleaved to yield acetic acid and isobutyric acid. Leucine 2,3-aminomutase is found in animal livers as well as in human leukocytes. The existence of leucine 2,3-aminomutase activity has now been established in bean seedlings and this may be the first report of a cobalamin-catalyzed activity in higher plants.

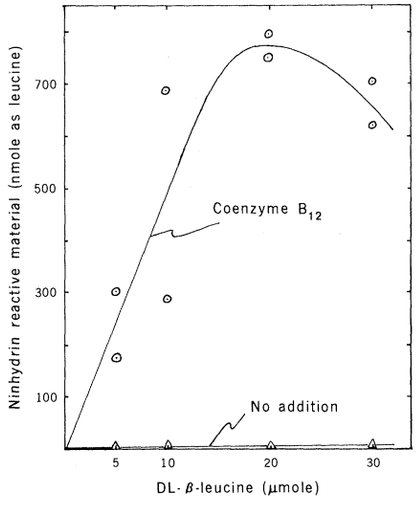

Fig. 1. Production of ninhydrin-reactive material from DL-β-leucine. Reaction conditions were those described in Table 1. After 60 minutes the reaction was stopped by the addition of 0.10 ml of 20 percent (weight to volume) of $HClO_4$. Portions (0.05 ml) of the clear deproteinized solution were taken for ninhydrin assay.

Navy beans (*Phaseolus vulgaris*) were sprouted on a bed of expanded vermiculite kept wet with distilled water and maintained at room temperature in the dark. After 5 days, the etiolated seedlings were harvested. The roots were washed free of vermiculite with distilled water and the plants were drained and weighed. The seedlings, together weighing 354 g, were homogenized in 250 ml of 0.1*M* potassium phosphate buffer, *p*H 7.0, by means of a Waring blender operating at top speed for 1.5 minutes. The homogenate was centrifuged at 16,000*g* for 40 minutes in a refrigerated centrifuge; 500 ml of cell-free extract was reserved for assay. The protein concentration of the extract was 20.3 mg per milliliter as estimated by the method of Lowry *et al.* (*2*).

Leucine 2,3-aminomutase activity was assayed by means of the reverse reaction. The α-leucine produced from the β-leucine substrate was measured by the increase in ninhydrin-reactive material or by gas chromatography after derivatization. Ninhydrin-reactive material was measured by the method of Rosen (*3*), and trimethylsilyl derivatives were made in an oil bath at 150°C, bis(trimethylsilyl)trifluoroacetamide (BSTFA) in acetonitrile (1 : 1 by volume) being used according to the method of Gehrke and Leimer (*4*). The derivatives were chromatographed on a column of 10 percent (by weight) OV-11 on 100/200 mesh Suplecoport (1.8 m by 2 mm) at a constant temperature of 110°C for 5 minutes followed by a temperature increase of 5°C per minute to a maximum temperature of 300°C.

Figure 1 shows the significant increase in the production of ninhydrin-reactive

Table 1. The conversion of β-leucine to α-leucine in extracts of *P. vulgaris*. The reaction mixtures (*1*) contained (in 1.99 ml) 100 m*M* triethanolamine-HCl buffer (*p*H 8.5), 0.5 μ*M* flavine adenine dinucleotide, 0.5 m*M* reduced coenzyme A, 0.5 m*M* nicotinamide adenine-dinucleotide, 0.5 m*M* pyridoxal phosphate, 1 mg of crude extract protein, and the indicated amounts of DL-β-leucine. When added, the concentration of coenzyme B_{12} was 3.4 m*M*. Reaction mixtures were incubated under argon at 30°C. After 60 minutes the reaction was stopped by the addition of 0.05 ml of 6*N* HCl followed by 0.10 ml of 10 percent (weight to volume) sodium tungstate. Portions (0.05 ml) were taken to dryness and derivatives were obtained with 0.1 ml of BSTFA and 0.1 ml of acetonitrile as described in the text. Portions (2 to 5 μl) were taken for gas chromatography.

DL-β-leucine added (μmole)	Actual α-leucine formed (nmole)	
	No addition	Coenzyme B_{12} added
0 (Control)	0	0
5	153	379
10	392	825
20	355	697
30	355	1327

material caused by the addition of coenzyme B_{12}. This result prompted an examination of the yield of α-leucine itself. Table 1 shows the results of another experiment in which the addition of coenzyme B_{12} caused a twofold increase in the yield of α-leucine as measured by direct, specific assay.

The effect of the corrin-binding mucoprotein, intrinsic factor, is shown in Table 2. The extract was prepared separately from that shown in Table 1, and its activity was greatly inhibited by the addition of intrinsic factor; the addition of coenzyme B_{12} prevented the effect of intrinsic factor. These results are consistent with the inhibition being caused by the effective removal of the corrinoid cofactor from the leucine 2,3-aminomutase.

The presence of a cobalamin-dependent enzyme in the bean is surprising, since it has long been supposed that plants make no corrin compounds, nor do they require them from exogenous sources. It is interesting to speculate that the apoenzyme is produced by the leguminous plant and the corrin cofactor is supplied by nodular bacteria. *Rhizobium meliloti* and *R. trifolii* have been shown to synthesize vitamin B_{12} 5 to 14 times as well when isolated from pink root nodules as when grown in vitro (*5*). There were no obvious nodules on the seedlings used in these experiments. In another experiment, the beans were surface-sterilized with sodium hypochlorite solution and sprouted on sterile vermiculite kept wet with sterile distilled water. Extracts prepared from these beans were as active as those prepared under nonsterile conditions. Bacterial plate counts from tissue of the aseptically sprouted beans showed this tissue to be free of bacteria while the nonsterile beans had up to 2×10^6 bacterial cells per gram of tissue. The endogenous activity shown in Table 1 may represent either cobalamin-independent activity or the supplying of cofactor to the seed from the nodules of the parent plant. It is possible that the natural cofactor is not a corrin compound, but is one for which added coenzyme B_{12} substitutes. This is a remote probability, for such cofactors have not been described.

It is unlikely that the activity noted in these extracts could have arisen from contaminating bacteria, since the percentage of protein that could be ascribed to bacterial sources was necessarily small. Bacteria grown from the nonsterile beans possessed the cobalamin-dependent mutase activity but did not release any of the activity into the soluble fraction when subjected to homogenization in a Waring blender; sonic disruption was required instead. This indicates that any contaminating bacterial cells would have been removed from the homogenized plant extracts along with the insoluble plant material.

Cobalamin-dependent leucine 2,3-aminomutase activity appears not to be limited to leguminous plants. Seed of the annual ryegrass (*Lolium* spp.) was sprouted in a manner similar to the beans and showed a similar level of activity.

Table 2. The effect of intrinsic factor on the activity of leucine 2,3-aminomutase in bean seeds. Reaction mixtures and conditions were as described in Table 1 except that 20 μmole of DL-β-leucine and 0.9 mg of crude extract protein were added; 0.1 mg of intrinsic factor was added where indicated. Zero time blank of 53 nmole has been subtracted from all values.

Addition	α-Leucine formed (nmole)
None	76
Coenzyme B_{12}	296
Intrinsic factor	14
Coenzyme B_{12} + intrinsic factor	295

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Plasma Membrane: Rapid Isolation and Exposure of the Cytoplasmic Surface by Use of Positively Charged Beads

Abstract. *Erythrocytes were ionically attached to polylysine-coated beads 30 micrometers in diameter. The binding of the cells was so tenacious that lysis or disruption of the attached cells left the beads covered by plasma membranes whose cytoplasmic surface was exposed and accessible for further analysis.*

Many of the properties of a plasma membrane are determined by its composition and its interactions with intracellular elements. Composition can be analyzed once the membrane is isolated and purified; interaction with intracellular elements can be assayed if the protoplasmic surface of the isolated membrane remains freely accessible after purification. We have devised a method of isolating cell membranes which readily yields purified plasma membranes whose protoplasmic surfaces are fully accessible to further probing. This method employs specially coated polyacrylamide or glass beads whose charged surfaces promote the nonspecific adherence of negatively charged cells (*1*). We test and illustrate its use to isolate the plasmalemma of the human erythrocyte because this membrane has been well characterized after conventional isolation procedures.

One milliliter of well-washed, packed human erythrocytes was added to 1 ml of packed polylysine-coated polyacrylamide beads 30 μm in diameter (Fig. 1). Because of their net negative charge (*2*), the erythrocytes, present in excess, adhered to and completely covered the positively charged, polylysine-coated beads. A sucrose-phosphate buffer of low ionic strength (Fig. 1, legend) favored the initial bead-cell interaction and promoted maximal coverage. Attraction between beads and cells was such that many cells bound two beads simultaneously (Fig. 1a), effectively agglutinating them. Be-