A METABOLITE OF α-AMINOADIPIC ACID IN YEAST: RELATION TO LYSINE BIOSYNTHESIS

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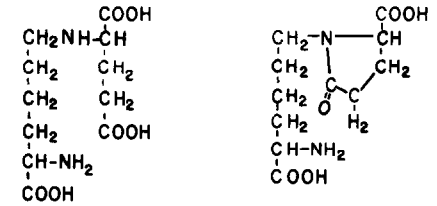
Although α-aminoadipic acid has been established as a precursor of lysine in yeast (Strassman and Weinhouse, 1953; Broquist et al, 1961a), and Neurospora (Mitchell and Houlahan, 1948; Windsor, 1951), the intermediate steps in its transformation to lysine are not known. α-Aminoadipic-δ-semialdehyde has been postulated as an intermediate (Yura and Vogel, 1959; Sagisaka and Shimura, 1959; and Broquist et al, 1961b), but the free semialdehyde has not been isolated. During a study of the metabolic fate of ε-C^{14}-DL-α-aminoadipic acid in resting yeast cells, an unknown radioactive compound, termed 'compound B', was formed. Upon continued incubation, the radioactivity of compound B decreased, with the concomitant appearance of radioactivity in lysine. A cell-free extract from yeast was subsequently obtained which efficiently converted α-aminoadipate to compound B but did not form lysine. Crystalline compound B was isolated from both the resting cell and cell-free systems and was found to be identical with L-saccharopine which has recently been isolated from yeast by Darling and Larsen (1961) and shown to be ε-N-(L-glutaryl-2)-L-lysine (Fig 1.) by Kjaer and Larsen (1961).

Separation of α-aminoadipic acid, compound B, and lysine was achieved by chromatography on a Dowex-1-formate column employing an ammonium formate gradient elution. Table I illustrates the formation of compound B from α-aminoadipate and subsequent lysine biosynthesis by resting yeast cells. It was possible to account for all of the administered ε-C^{14}-DL-α-aminoadipic acid radioactivity. After thirty minutes incubation, half of the initial α-aminoadipic acid had disappeared from the medium; of the radioactivity in the cells, 90% was

accounted for as compound B while only 10% of the radioactivity appeared in lysine. If the residual radioactivity in the medium is due to D-α-aminoadipic acid, as seems likely, the synthesis of compound B in this system is extremely efficient. After 2 hours incubation, the radioactivity of compound B decreased to 40% of the total activity in the cells with a corresponding rise in radioactivity of lysine to 60%.



L-Saccharopine Compound B

L-Pyrosaccharopine

Fig. 1. L-Saccharopine, compound B, and L-pyrosaccharopine structures.

A cell-free yeast extract which forms compound B from α-aminoadipate was prepared as follows. One pound of Fleishman's yeast was frozen and thawed several times in liquid nitrogen. The homogenate was extracted for 2 hours with 0.1 M phosphate buffer, pH 7.5, on a shaker at 0°C. The mixture was centrifuged at 10,000 x g for 20 minutes to remove the cell debris, and the turbid supernatant was lyophilized immediately (yield, 30.5g). Two grams of the lyophilized material was dissolved in 0.02 M phosphate buffer, pH 7.5, and treated with Sephadex G-50 (Pharmacia Co.) to separate the proteins from compounds of low molecular weight (Kisliuck, 1960). These latter fractions were lyophilized and served as a source of enzyme and "cofactors", respectively. The experiment described in Table 2 indicates that synthesis of compound B is dependent on α-aminoadipate, "cofactors", and enzyme.

The enzyme system described above was used to prepare large quantities of compound B for chemical characterization. The deproteinized reaction mixture was purified by column chromatography using the following sequence: Dowex-1-formate, eluted with an ammonium formate gradient; Dowex-50 (hydrogen form),

TABLE I

Time Sequence Study of Lysine Formation From DL-α-Aminoadipic Acid in Resting Cells of Saccharomyces cereviseae

Experimental: To 17 ml 0.1M pH 3.5 phosphate was added 2.2 μc ε-C^{14}-DL-α-aminoadipic acid, 175 mg glucose, 2 gm (wet weight) S. cereviseae cells+, and the suspension vigorously shaken at 15°C. Aliqouts were removed at appropriate time intervals, and the cell suspensions centrifuged immediately at 2°C. Radioactivity in the supernatant was determined** and ascribed solely to residual α-aminoadipate, since chromatographic procedures revealed this amino acid as the only radioactive component in this fraction.

The cells were washed, frozen and thawed six times with liquid nitrogen, and finally treated with cold perchloric acid. The suspension was then centrifuged, perchloric acid removed from the supernatant by precipitation as the potassium salt, and the extract which contained free lysine and compound B chromatographed on a Dowex-1-formate column. The perchloric acid precipitate together with the cell debris was acid hydrolyzed (6N HCl, 110°C, 36 hrs) to release protein-bound lysine, and the hydrolyzate chromatographed on a separate Dowex-1-formate column. Radioactivity in the column eluates appropriate for compound B and total lysine was determined**.

Incubation time (min.)	Radioactivity*			
	In medium	In cells		
	α-aminoadipate	"B"	Lysine	Lysine + "B"
0	1.00	-	-	
30	0.498	0.424	0.048	0.472
60	0.482	0.225	0.258	0.483
120	0.510	0.205	0.304	0.509
180	0.505	0.156	0.306	0.462

* Expressed as: $\frac{\text{radioactivity found}}{\text{initial radioactivity of } \alpha\text{-aminoadipate}}$

\+ 18 hr. cells of Saccharomyces cereviseae (Y-80, a prototrophic strain, provided by Dr. H. Tresnor, Lederle Laboratories, Inc.) grown on glucose-tryptone-yeast extract medium.

** Radioactivity of yeast fractions was determined by drying an aliqout of the sample on a metal planchet and counting in a windowless Gas-flow counter.

eluted with 1N ammonium hydroxide; Dowex-1-formate, eluted with a formic acid gradient. The final eluate, after treatment with activated charcoal, yielded crystalline compound B with constant specific radioactivity from water: ethanol mixtures at pH 3.5. The same procedure was used to isolate crystalline compound B synthesized by whole cells. A comparison of the infra-red spectra

showed that the crystals obtained from the whole cell experiment and from the enzymic reaction were identical. Elemental analysis gave: C = 47.17%; H = 7.57%; N = 10.07%; calculated for $C_{11}H_{20}N_2O_6$: C = 47.82%; H = 7.30%; N = 10.14%.

TABLE 2

Enzymatic Formation of Compound B From ϵ-C^{14}-DL-α-Aminoadipic Acid in a Partially Purified Cell-Free Extract of Baker's Yeast.

The additions shown in the table, at pH 7.0 were shaken for 2 hrs. at 30°C. The reaction mixtures were then deproteinized by treatment with perchloric acid and centrifuged. The clear supernatants were spotted on Whatman No. 1 paper and chromatographed in a butanol-acetic acid-water (4:1:1) system by descending chromatography.

Additions per ml.			Radioactivity found in compound B (counts/min**)
α-aminoadipate+	cofactors*	enzyme*	
+	122mg	-	0
+	"	8.5mg	93
+	"	17.0	152
+	"	25.5	300
+	"	34.0	538
+	-	34.0	2
+	-	crude***	628

\+ DL-α-aminoadipic acid, 30 µmoles per ml, containing 10,000 cpm as ϵ-C^{14}-DL-α-aminoadipic acid.

** The radioactive spot corresponding to "B" was cut out, and counted in a windowless gasflow counter.

*** Sephadex treatment omitted.

* See text.

Compound B is an optically active, ninhydrin positive amino acid which melts with decomposition at 242°-244°C. The compound has four pK values, 2.5, 3.9, 9.2, and 10.2, indicating the presence of at least two nonequivalent amines and acidic groupings. Compound B and saccharopine can not be

distinguished from one another by paper chromatography, and they exhibit identical infra-red spectra and X-ray diffraction patterns. On heating compound B with 6N HCl for 24 hours at 110°C, a new ninhydrin positive amino acid was formed. This degradation product, subsequently isolated in crystalline form, gave an infra-red spectrum and melting point identical to those of L-pyrosaccharopine (Fig 1), which Kjaer and Larsen (1961) had obtained from L-saccharopine by similar procedures.

Compound B does not support the growth of the lysine auxotroph, Neurospora crassa (33933), which is able to utilize α-aminoadipate in lieu of lysine; however compound B may be unable to penetrate such cells. Detailed studies of the manner in which compound B is formed from α-aminoadipic acid and subsequently converted to lysine by suitable cell-free enzyme systems are in progress.

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