THE SEPARATION OF THE ASPARTYL- AND ASPARAGINYL- RNA SYNTHETASES OF LACTOBACILLUS ARABINOSUS

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The purification of aspartyl-RNA synthetase from extracts of Lactobacillus arabinosus has recently been reported (Norton, et al., 1963). Since there is little information available on an amino acyl-RNA synthetase which is specific for asparagine, it was of interest to determine whether extracts of L. arabinosus contain an asparaginyl-RNA synthetase, and, if so, whether this activity can be separated from the aspartyl-RNA synthetase activity. In the present investigation, it was found that extracts of L. arabinosus catalyze the transfer of C^{14}-asparagine to RNA and that this activity can be separated from the aspartyl-RNA synthetase activity.

EXPERIMENTAL

Materials.--Uniformly labeled C^{14}-L-asparagine (specific activity, 22.5 mc/mmole) was obtained from Nuclear-Chicago Corporation. Uniformly labeled C^{14}-L-aspartic acid (specific activity, 125 mc/mmole) obtained from Schwarz BioResearch, Inc. was diluted with chromatographically pure C^{12}-L-aspartic acid obtained from Mann Research Laboratories to a specific activity of 20 mc/mmole. Soluble RNA from Escherichia coli was obtained from General Biochemicals, and the DEAE-cellulose, type 20, was obtained from Brown and Company.

Enzyme Assays.--To measure asparaginyl-RNA synthetase activity, the reaction mixture contained: C^{14}-L-asparagine, 10 mμmoles containing 210,000

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c.p.m.; K_2ATP, 0.4 μmoles; Tris buffer, 50 μmoles; magnesium chloride, 15 μmoles; E. coli soluble RNA, 1 mg; and a rate-limiting amount of enzyme, in a total volume of 0.5 ml at a pH of 7.5. To measure aspartyl-RNA synthetase activity, the reaction mixture was modified by substituting 10 mμmoles of C^{14}-L-aspartic acid containing 160,000 c.p.m. for the asparagine and by increasing the concentration of the K_2ATP to 4 μmoles. The reaction mixtures were incubated for 15 minutes at 37°. The reaction was terminated by the addition of 2.5 ml of cold 67% ethanol containing 0.5 M sodium chloride, and the RNA was precipitated and washed three times as previously described (Berg, et al., 1961). The washed RNA was dissolved in 1 ml of water, a 0.5 ml aliquot was plated and dried, and the radioactivity was measured in a windowless gas-flow counter. Proteins were determined by the method of Lowry et al. (1951). Specific activities are reported as mμmoles of amino acid transferred to RNA per mg of protein per hour.

Enzyme Purification Procedure.--Cells of L. arabinosus 17-5 (ATCC 8014), grown in 2 liters of yeast extract-peptone medium (McMahan and Snell, 1944) supplemented with 3 ml of Tween 80 per liter, were harvested after 16 hours incubation at 30°. The cells were washed and resuspended in 70 ml of 0.04 M Tris buffer, pH 8.0, containing 0.01 M 2-mercaptoethanol, and the cell suspension was subjected in 35-ml portions to sonic oscillation in a Raytheon 10 kc oscillator for 30 minutes. Centrifugation of the disrupted cell suspension at 4° for 1 hour at 105,000 x g provided a clear cell-free extract containing approximately 11 mg of protein per ml. All subsequent steps in the fractionation procedure were carried out at 4°, and all buffer solutions contained 0.01 M 2-mercaptoethanol.

Cold, saturated ammonium sulfate solution, which had been brought to pH 7 by the addition of ammonium hydroxide, was added to the cell extract to bring the concentration of ammonium sulfate to 56, 66 and 76 per cent of saturation. The precipitates were removed by centrifugation and resuspended in 0.04 M Tris buffer, pH 7.5.

A column 1 x 16 cm in size was prepared with 1.6 g of DEAE-cellulose which had been equilibrated with 0.04 M Tris buffer, pH 7.5. A portion of the 66-76 per cent ammonium sulfate fraction, containing 66 mg of protein, was dialyzed for two hours against 0.04 M Tris buffer, pH 7.5, and passed through the DEAE-cellulose column. The column was washed with 32 ml of the same buffer, followed by 32 ml of 0.04 M Tris buffer, pH 7.5, containing 0.1 M potassium chloride. The adsorbed protein was eluted step-wise with 16 ml portions of 0.04 M Tris buffer, pH 7.5, containing 0.15, 0.2, 0.25, 0.3 and 0.35 M potassium chloride. The active fractions could be stored at 4° up to one week without appreciable loss in activity if bovine serum albumin (1 mg per ml) were added to the fractions.

RESULTS AND DISCUSSION

As shown in Table I, crude cell extracts of L. arabinosus catalyze the transfer of both aspartic acid and asparagine to RNA. Some aspartyl- and asparaginyl-RNA synthetase activity was recovered in the 56-66 per cent ammonium sulfate fraction; however, the 66-76 per cent ammonium sulfate fraction appeared to contain more of the asparaginyl-RNA synthetase activity. Attempts to further purify the 66-76 per cent ammonium sulfate fraction by adsorption and elution from calcium phosphate gel, which was the procedure previously described for the purification of the aspartyl-RNA synthetase, resulted in considerable loss in ability to transfer asparagine to RNA. Additional experiments indicated that phosphate ion strongly inhibits the transfer of asparagine to RNA. Adsorption of the 66-76 per cent ammonium sulfate fraction on DEAE-cellulose and elution with potassium chloride gave not only a good recovery of the asparaginyl-RNA synthetase but also a good separation from aspartyl-RNA synthetase. The aspartyl-RNA synthetase is eluted from the DEAE-cellulose column by 0.2 M potassium chloride (Fraction 3) while the asparaginyl-RNA synthetase is not eluted from the column until the concentration of potassium chloride is increased to 0.35 M (Fraction 6).

Table I

Separation of Asparaginyl-RNA Synthetase from Aspartyl-RNA Synthetase

Fraction	Aspartyl-RNA Synthetase		Asparaginyl-RNA Synthetase	
	Yield %	Specific Activity*	Yield %	Specific Activity*
Cell-free extract	100	1.4	100	0.28
Ammonium sulfate fraction, 66-76%	40	3.2	57	1.1
Elutions from DEAE-cellulose column				
Fraction 3 (0.2 M KCl)	23	21.4		< 0.1
Fraction 6 (0.35 M KCl)		< 0.1	34	14.8

*mμmoles amino acid transferred/mg protein/hr.

Table II

Dilution of C^{14}-Aspartate and C^{14}-Asparagine Transfer to RNA by C^{12}-Aspartate and C^{12}-Asparagine

Additions to Reaction Mixture*	C^{14}-Amino Acid Transferred to RNA	
	DEAE Fraction 3	DEAE Fraction 6
	c.p.m.	
C^{14}-Aspartate, .01 μmole	1233	32
C^{14}_{12}-Aspartate, .01 μmole + C^{12}-Aspartate, 1.0 μmole	99	--
C^{14}_{12}-Aspartate, .01 μmole + C^{12}-Asparagine, 1.0 μmole	1423	--
C^{14}-Asparagine, .01 μmole	54	1160
C^{14}_{12}-Asparagine, .01 μmole + C^{12}-Aspartate, 1.0 μmole	--	1138
C^{14}_{12}-Asparagine, .01 μmole + C^{12}-Asparagine, 1.0 μmole	--	12

*The other components of the reaction mixture and the assay procedure were the same as described in the Experimental Section. The amounts of enzyme added were 33 μg protein from DEAE fraction 3 and 26 μg protein from DEAE fraction 6. The c.p.m. are corrected for "zero time" controls which average between 50 and 150 c.p.m.

As shown in Table II, Fraction 3 catalyzes the transfer of C^{14}-aspartic acid but not C^{14}-asparagine to RNA. Almost complete dilution of the transfer of C^{14}-aspartic acid to RNA is observed in the presence of C^{12}-aspartic acid, and no dilution is observed in the presence of C^{12}-asparagine. Fraction 6, on the other hand, catalyzes the transfer of C^{14}-asparagine but not C^{14}-aspartic acid to RNA. Complete dilution of the transfer of C^{14}-asparagine to RNA by this fraction is observed in the presence of C^{12}-asparagine, and no dilution is observed in the presence of C^{12}-aspartic acid.

From these data, it can be concluded that two enzymes are required for the transfer of aspartic acid and asparagine to RNA, one of which is specific for aspartic acid and the other specific for asparagine.

References

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