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Cell preparation for the assay of threonine dehydratase in *Escherichia coli*

Threonine dehydratase (L-threonine hydro-lyase (deaminating), EC 4.2.1.16, formerly known as threonine deaminase) is the initial enzyme of the biosynthetic pathway leading to L-isoleucine in *Escherichia coli*. In previous studies on this enzyme, we reported[1] that the activity in sonic extracts was extremely labile even at 0°. Furthermore, its activity is usually determined in extracts prepared by sonic treatment of cells[1–5]. In this communication, the loss in enzyme activity of biosynthetic threonine dehydratase by sonic treatment is reported and, in view of this drawback, a better method of cell preparation for threonine dehydratase assay is suggested. Of the methods investigated, shaking with toluene gave the maximum activity in *E. coli* preparations.

E. coli K-12 (wild type) was grown with vigorous aeration in glucose–salts medium[1]. The cells were harvested in mid-log phase by centrifugation at 6000 × *g* for 10 min in a refrigerated Sorvall centrifuge. The packed cells were resuspended in 0.1 M phosphate buffer (pH 8.0) to give a suspension of the required cell density. Prior to enzyme assays, the extracts were prepared as indicated in each table. The assay system consisted of the following in a total volume of 1 ml: 0.5 ml enzyme extract, 80 μmoles L-threonine, 100 nmoles pyridoxal phosphate, and 100 μmoles sodium phosphate (pH 8.0). The extract was incubated at 37° with the above reaction mixture and after 15 min the reaction was stopped by the addition of 0.1 ml of 50% trichloroacetic acid. The volume in each tube was made up to 3 ml prior to determination of

TABLE I

METHODS OF CELL DISRUPTION AND THREONINE DEHYDRATASE ACTIVITY OF *E. coli* K-12

Method of disruption		*Specific activity*
1. Sonic treatment, 2 min (20-kcycle Bronwill sonic oscillator)		2.9*
2. Repeated freezing and thawing (3–10 ×)		6.8*
3. Treatment with surface active agents		
(a) Toluene	0.5%	17.3*
(b) Triton	0.8%	1.5
(c) Tween 80	0.8%	0.3
(d) Sodium desoxycholate	0.2%	1.1
(e) Sodium lauryl sulfate	0.2%	1.7
(f) "Duponol"	0.2%	2.1
4. Treatment with lysozyme and EDTA		5.4

* Average of about 20 independent experiments.

α-ketobutyric acid by the method of FRIEDEMANN[6]. The specific activity of the enzyme was expressed as μmoles of keto acid formed per mg of protein per h. Protein was determined by the method of LOWRY *et al.*[7].

Table I shows that the highest threonine dehydratase activities were obtained when cells were treated with toluene. Lower activities were obtained by alternate

TABLE II

EFFECT OF CELL DENSITY ON THREONINE DEHYDRATASE ACTIVITY OF SONIC-DISRUPTED AND TOLUENE-TREATED PREPARATION OF *E. coli* K-12

Sonic extract: treated for 2 min at 20 kcycles. Toluene extract: 0.5% toluene.

Cell density (mg protein/ml)	*Specific activity*	
	Sonic extract	*Toluene extract*
0.1	—	24.5
0.2	3.1	—
0.4	6.4	—
0.5	—	24.6
0.8	11.0	—
1.0	—	25.3
1.6	11.6	—
3.2	13.2	—

TABLE III

LOCALIZATION OF THREONINE DEHYDRATASE ACTIVITY IN SONIC AND TOLUENE EXTRACTS OF *E. coli* K-12

Fraction	*Distribution (%)*			
	Sonic extract		*Toluene extract*	
	Protein	*Enzyme activity*	*Protein*	*Enzyme activity*
Whole extract	100	100	100	100
Centrifugation at 18 000 × *g*				
(a) Supernatant	62	40	6	0
(b) Pellet	44	48	95	100
Specific activity		2.9		17.3

TABLE IV

PREPARATION OF CELL EXTRACT BY TOLUENE TREATMENT FOR THREONINE DEHYDRATASE ASSAY

Toluene was added to ice-cold suspension and shaken gently for 2 min prior to enzyme assay.

Toluene concn. (%)	*Specific activity*
0	0.9
0.05	3.7
0.1	16.4
0.5	17.6
1.0	15.3

freezing and thawing, by sonic treatment, or by treatment with a variety of surface active agents or lysozyme. It was observed also that cell density was an important factor when sonic treatment was employed but not when toluene treatment was used (Table II). As shown in Table III, when sonic extracts were centrifuged at 18 000 × *g* for 30 min, the pellet contained about 50% of the enzyme activity. In contrast, all of the enzyme activity of the toluenized preparations was found in the 18 000 × *g* pellet. The procedure finally adopted in order to obtain maximum activities consisted of gentle shaking for 2 min at 0° with 0.5% (v/v) of toluene (Table IV), followed immediately by determination of the enzyme as outlined. This procedure usually gives about 5–10 times higher threonine dehydratase activity than that obtained by sonic treatment.

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Manganese activation of a (Na^+-K^+)-dependent ATPase in pig brain microsomes

ATPase (ATP phosphohydrolase, EC 3.6.1.3) activity, which is dependent on Mg^{2+} and is activated by Na^+ and K^+, has been reported in membrane preparations from numerous tissues of higher animals[1–4]. These (Mg^{2+}-Na^+-K^+)-ATPases are inhibited by ouabain, which also inhibits the active transport of Na^+ and K^+ in many tissues. Membrane (Na^+-K^+)-activated ATPases are, therefore, believed to be part of the mechanism pumping Na^+ out of, and K^+ into, cells, particularly nerve cells.

In at least some enzymes involved in the metabolism of ATP, such as pyruvate kinase, Mn^{2+} can be substituted for Mg^{2+} as divalent metal activator[5,6]. This paper presents evidence that pig brain microsomal ATPase activity can be activated when Mn^{2+} replaces Mg^{2+}, and that this activity is enhanced by the joint addition of Na^+ and K^+.