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Abstract

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Tateson, R.W. A method for the determination of arginosuccinate synthetase.

A method for determining arginosuccinate synthetase (L-citrulline: L-aspartate lyase (AMP) 6.3.4.5.) by measuring the production of arginosuccinic acid (ASA) has been developed in this laboratory. This has been found to be more reliable and accurate than methods measuring the rate of loss of citrulline in the presence of large amounts of citrulline. To prevent the destruction of ASA, by the enzyme ASAase, fumarate is included in the reaction mixture which keeps the reaction: ASA $\rightleftharpoons$ arginine + fumarate well over to the left by a mass action effect. As ATP is inhibiting in high concentrations, a low concentration is used, plus two ATP generating systems.

The following stock solutions are made up: CIT = 0.986 g L-citrulline in 25 ml water; ASP = 1.00 g L-aportic acid in 100 ml water; FUM = 16.0 g No fumarate in 100 ml water; Mg = 4.94 g $MgSO_4.7H_2O$ in 50 ml 1 M Tris; TRIS = 1 M Tris; PGA = 4.46 g Ba D(-)3-phosphoglyceric acid.$2H_20$ in 75 ml 1 N HCl (odd 20 ml saturated K_2SO_4, spin off ppt., make up to 100 ml); ATP = 0.031 g ATP (disodium salt) in IO ml water. All solutions ore adjusted to pH 7.4 before being mode up to final volume. All solutions may be stored frozen.

The reaction mixture consists of:

Solution	Vol. ml.	µmoles in 0. 1 ml rxtn. mixture
CIT	1.0	2.25
ASP	3.0	2.25
Mg	0.5	2.58
TRIS	0.5	10.0
FUM	I.0	15.0
PGA	4.0	5.0
to this is added:		
Creatine phosphate	0.127 g	6.0
Creatine phaphokinose (Sigma London Ltd.)	0.001 g	

The enzyme is prepared by homogenizing 40 g of freeze-dried Neurospora powder in 1 ml of 0.05 M Tris pH 7.5 buffer, and centrifuging at 3000 rpm for 5 min. The supernatant is dialyzed against two changes of 0.05 M Tris pH 7.5 at 4°C.

0. 1 ml of enzyme extract is added to 0. 1 ml of reaction mixture in a small centrifuge tube and allowed to equilibrate at 35°C. The reaction is started by adding 0. 1 ml of ATP solution. For each assay three tubes ore made up and the reaction is stopped after 0, 30 and 60 min by adding 0.05 ml of 5% TCA. The tubes ore boiled for 15 min and then spun at 3000 rpm for a short time.

0.05 ml of the supernatant ore spotted onto 3MM chromatography paper which is subjected to electrophoresis in a pyridine-acetic acid buffer pH 3.7 for 60 min at 3000 volts, in on Anfinson-type tank. The papers are developed by the method of Bronk and Fisher (1956 Biochem. J. 64: 106). ASA runs as two spots, as it is converted into its onhydrides by boiling with acid. These spots are cut out, eluted with 2 ml methanol, and the $OD_{504\ m\mu}$ measured. The OD is proportional to time and protein concentration. ▪ ▪ ▪ Department of Genetics, University of Edinburgh, West Mains Road, Edinburgh 9, Scotland.