

Determination of arginase: estimation of citrulline

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Abstract

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Morgan, D. H. The assay of arginase.

Many methods of arginase assay in various organisms have been published. The following procedure has been found to work well with crude extracts of *Neurospora*. Frozen mycelial pods are ground in a chilled mortar with glass powder and 5-10 times their weight of pH 7.0 0.025 M maleic acid/sodium hydroxide buffer containing manganese chloride (0.005 M) and dithioerythritol (0.002 M). An -SH reagent is possibly superfluous in undialysed extracts but has been found to stabilize the enzyme during dialysis.

A reaction mixture consists simply of 0.2 ml of enzyme and 0.3 ml of 0.3 M arginine. The arginine solution is adjusted to pH 9.5 and final pH in the mix is about 9.2, the arginine itself providing adequate buffering. Incubation is at 37°C, the enzyme being pre-incubated at this temperature for 10-15 min before arginine addition. The reaction is stopped with 4.5 ml of 2% TCA, zero-time blanks being stopped before arginine addition. Ornithine estimation (see below) is carried out on 0.5 ml samples of the stopped reaction mix. (When it is desired to use lower substrate concentrations and therefore to detect lower levels of ornithine, to stay in the linear region dilution with TCA is reduced or avoided altogether by stopping with the acid ninhydrin reagent used for the ornithine estimation.)

The estimation of ornithine in assaying for arginase or acetyl-ornithine/glutamate transacetylase. The method of ornithine estimation used by Vogel and Bonner (1956 J. Biol. Chem. 218:97) for the assay of acetylornithinase is also applicable to the assay of arginase and acetylornithine/glutamate transacetylase. It is quicker than the commonly-used method of Chinard (1952 J. Biol. Chem. 199: 91) and the ninhydrin mix used (made up in 0.4 M citric acid and methyl cellosolve) is pleasanter to deal with than that of Chinard (6 M phosphoric acid and glacial acetic acid). The optimum boiling time for arginase samples is 25 min. Both glutamate and arginine give rise to a deep blue color after the final addition of NaOH. This persists until the samples are subjected to vigorous vortex mixing (30-60 sec) when it disappears, revealing the stable golden-brown color which is read at 470 m μ . It is necessary to read against no-ornithine blanks containing appropriate quantities of arginine or glutamate, both of which give appreciable blank values. Sensitivity is about three-fold lower than with Chinard = 0.4 μ moles of ornithine per OD unit at 470 m μ in the presence of 9 μ moles arginine per sample. The reaction is linear at least up to 0.5 μ moles ornithine per sample.

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