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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 hove 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 gloss powder and 5-10 times their weight of pH 7.0 0.025 M moleic 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 ond 0.3 ml of 0.3 M orginine. The orginine solution is adjusted to pH 9.5 and final pH in the mix is about 9.2, the orginine itself providing adequate buffering. Incubation is at 37°C, the enzyme being pre-incubated at this temperature for IO-15 min before arginine addition. The reaction is stopped with 4.5 ml of 2% TCA, zero-time blanks being stopped before orginine 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 ond 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 tronsacetylose. The method of ornithine estimation used by Vogel and Bonner (1956 J. Biol. them. 218: 97) for the ossoy of acetylornithinase is also applicable to the assay of arginase and acetylornithine/glutamate tronsacetylose. It is quicker than the commonly-used method of Chinord (1952 J. Biol. Chem. 199: 91) ond the ninhydrin mix used (mode up in 0.4 hl citric acid and methyl cellosolve) is pleasanter to deol with than that of Chinord (6 M phosphoric acid ond glacial acetic acid). The optimum boiling time for arginase samples is 25 min. Both glutamate and orginine give rise to a deep blue color after the final addition of NaOH. This persists until the samples ore subjected to vigorous Vortex mixing (30-60 sec) when it disoppeors, revealing the stable golden-brown color which is read at 470 mμ. It is necessary to read against no-ornithine blanks containing appropriate quantities of orginine or glutamate, both of which give appreciable blank values. Sensitivity is about three-fold lower than with Chinard ▪ 0.4 μmoles of ornithine per OD unit at 470 mμ in the presence of 9 μmoles orginine per sample. The reaction is linear at least up to 0.5 μmoles ornithine per sample.

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