

Adenylosuccinate lyase determination

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Abstract

Adenylosuccinate lyase determination

Tateson, R. W. A method for the determination of adenylosuccinate lyase.

The reported method for this enzyme (Adenylosuccinate AMP lyase 4.3.2.2) required a supply of adenylosuccinate (Giles et al. 1957 Proc. Natl. Acad. Sci. U.S. 43:305). As adenylosuccinate is not available commercially, a method has been devised to measure the back reaction whose substrates, fumarate and AMP, are readily available.

The enzyme was prepared in the same way as for the ASA synthetase below. The reaction: Into a 1 cm. cuvette are added 0.1 ml of 0.01 M AMP, 0.05 ml of 0.05 M sodium fumarate, 2.75 ml of 0.1 M phosphate/citrate buffer pH 7.5, and 0.1 ml enzyme extract. The increase in $OD_{280m\mu}$ is measured at 35°C in a recording spectrophotometer against a blank containing no AMP. There is some change in OD in the blank due to the activity of fumarase, but this is kept very low by the citrate buffer, citrate being an inhibitor of fumarase. The reaction is linear for 10 min. and is proportional to protein concentration over a 50-fold range. - - - Department of Genetics, University of Edinburgh, West Mains Road, Edinburgh 9, Scotland.