Adenylosuccinate lyase determination

R. W. Tateson

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

The reported method for this enzyme (Adenylsuccinate AMP lyase 4.3.2.2) required a supply of adenylsuccinate (Giles et al. 1957 Proc. Natl. Acad. Sci. U.S. 43:305). As adenylsuccinate 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 d-sodium fumarate, 2.75 ml of 0.1 M phosphate/citrate buffer pH 7.5, and 0.1 ml enzyme extract. The increase in absorbance is measured at 280 m\(\mu\) 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 Rood, Edinburgh 9, Scotland.

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: 

\[\text{ASA} \rightarrow \text{arginine} + \text{fumarate} \]

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-aspartic acid in 100 ml water; FUM = 16.0 g Na fumarate in 100 ml water; Mg = 4.94 g MgSO\(_4\).7H\(_2\)O in 50 ml 1 M Tris; TRIS = 1 M Tris; PGA = 4.46 g Na D(-)-3-phosphoglyceric acid.2H\(_2\)O in 75 ml 1 N HCl (add 20 ml saturated K\(_2\)SO\(_4\), spin off ppt., make up to 100 ml); ATP = 0.031 g ATP (disodium salt) in 10 ml water. All solutions are adjusted to pH 7.4 before being made up to final volume. All solutions may be stored frozen.

The reaction mixture consists of:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Vol. ml.</th>
<th>µmoles in 0.1 ml rxn. mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIT</td>
<td>1.0</td>
<td>2.25</td>
</tr>
<tr>
<td>ASP</td>
<td>3.0</td>
<td>2.25</td>
</tr>
<tr>
<td>Mg</td>
<td>0.5</td>
<td>2.58</td>
</tr>
<tr>
<td>TRIS</td>
<td>0.5</td>
<td>10.0</td>
</tr>
<tr>
<td>FUM</td>
<td>1.0</td>
<td>15.0</td>
</tr>
<tr>
<td>PGA</td>
<td>4.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

To this is added:

- Creatine phosphate 0.127 g
- Creatine phophokinase 0.001 g
- (Sigma London Ltd.)


The reaction mixture contained 0.16 µmole pyridoxal phosphate, 3 µmole magnesium sulphate, 0.06 µmole kynurene, 50 µmole Tris-(hydroxymethyl) aminomethane pH 8.0, and protein (50-500 µg) in a final volume of 1.2 ml. The reaction was conducted at 30°C in the cuvette of an Aminco Bowman spectrophotofluorimeter equipped with thermal spacer. Anthranilic acid which appeared during the reaction was detected by measuring the intensity of its fluorescence at 409 nm. Activation was at 315 nm.

The amount of anthranilic acid produced is linear with time for about 5 minutes, after which the rate decreases. The velocity of the reaction is proportional to protein concentration from 0.045 to 0.54 mg/ml and extrapolates to zero at zero protein concentration. The velocity of the reaction approached a maximal value when the kynurene concentration was 0.05 µmole/ml. Kynure-