Biosynthesis of the Purines

XXV. THE ENZYMATIC CLEAVAGE OF N-(5-AMINO-1-RIBOSYL-4-1MIDAZOLYL-CARBONYL)-L-ASPARTIC ACID 5'-PHOSPHATE*

RICHARD W. MILLER,† LEWIS N. LUKENS,‡ AND JOHN M. BUCHANAN

From the Division of Biochemistry, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts

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Succino-AICAR has been previously implicated as an intermediate in the pathway of purine biosynthesis de novo in extracts of avian liver (1, 2). This compound is formed when AIR is incubated with ATP, bicarbonate, magnesium ions, and aspartic acid in the presence of the appropriate fraction from pigeon or chicken liver (3). Succino-AICAR is cleaved by another enzyme which is present in soluble preparations of avian liver and also in extracts of Escherichia coli, Neurospora crassa, Salmonella typhimurium, human liver, beef liver, and bakers' yeast. The products of the cleavage of succino-AICAR were shown by Lukens and Buchanan (2) to be AICAR and a mixture of fumaric and malic acids, when crude enzyme preparations were employed.

The present paper describes the purification and properties of the cleaving enzyme from chicken liver and bakers' yeast and provides evidence for the positive identification of fumaric acid as the dicarboxylic acid which is initially formed when succino-AICAR is converted to AICAR (Reaction 1).

Due to the apparent structural similarities between succino-AICAR, adenylosuccinic, and argininosuccinic acids, the specificity of the cleaving enzyme with respect to these compounds was investigated. Several lines of evidence lead to the conclusion that the cleaving enzyme may be identical with adenylosuccinase as purified by Carter and Cohen (4).

EXPERIMENTAL

Materials

AICAR was prepared in quantities of 0.3 mmole by the method of Flaks et al. (5). Succino-AICAR was prepared by the method of Lukens and Buchanan (3). For large scale preparations, however, it was found more convenient to convert AICAR to succino-AICAR by reversal of the cleaving reaction. In one experiment 138 pmoles of purified AICAR were incubated with 1.35 mmoles of sodium fumarate and 18 units of the most highly purified enzyme fraction from yeast in a final volume of 15 ml. The reaction was carried out at 37° for 4 hours after which time the mixture was heated in a boiling water bath for 2 minutes and chilled. Succino-AICAR was obtained in about 80 per cent yield in this way, based on the disappearance of AICAR. In order to free the succino-AICAR from impurities the solution was diluted to 500 ml. with water and passed through a column of Dowex 1 ion exchange resin, 10 per cent cross-linked, in the bromide form. The dimensions of the column used were 15 x 2 cm. After washing the column with water, succino-AICAR was eluted with 0.008 N HBr, the elution being followed by ultraviolet absorption and the modified Bratton and Marshall assay (3). In this way succino-AICAR was freed from AICAR and from ADP if the AICAR used in the reaction contained only traces of ADP.

Generous gifts of adenylosuccinic acid were obtained from Dr. C. E. Carter. This compound was also synthesized from fumaric acid and AMP by the method of Carter and Cohen (4). Argininosuccinic acid was the generous gift of Dr. S. Ratner.

Preparation of Succino-AICAR Cleaving Enzyme from Liver—
The cleaving enzyme was purified several fold from chicken liver...
with the specific intention of removing the contaminating enzyme, fumarase. Chilled chicken livers were minced and homogenized in a Waring Blender in a solution containing 35 parts by volume of 0.1 M K₂HPO₄, pH 7.5 and 45 parts of distilled water. The insoluble fraction was removed by centrifugation in the Stock centrifuge (31. capacity) at 0° for 3 hours at 3,500 × g. The cloudy supernatant fluid was fractionated with 90 per cent ethanol. The fraction precipitating between 30 and 45 per cent ethanol at −35° was collected by centrifugation in the Stock centrifuge at 5,300 × g for 1 hour. The precipitate was taken up in distilled water and lyophilized to dryness. The powder, which was obtained in a yield of 28 g., could be stored indefinitely at −20° without loss of enzymatic activity.

For further purification 3 g. of the lyophilized powder were taken up in 100 ml. of cold 0.05 m potassium acetate buffer, pH 5.4, and heated in a constant temperature bath at 53° for 3.5 minutes. The suspension was then rapidly cooled in a −5° ice bath and the resulting coagulum removed by centrifugation in the cold. For each ml. of clear supernatant solution 1.5 mg. of centrifuged calcium phosphate gel were then added. After centrifugation the supernatant solution was fractionated with 90 per cent ethanol. The fraction precipitating between 20 and 45 per cent ethanol was collected by centrifugation, dissolved in distilled water, and lyophilized. The dry powder was taken up in 0.05 m potassium acetate buffer, pH 5.4, to give a protein concentration of 25 mg. per ml. This concentrated solution was heated in a water bath at 59° for 1.5 minutes and then rapidly cooled. The coagulum was removed by centrifugation. The second heat step resulted in some loss of enzymatic activity but was retained because of its effectiveness in causing the destruction of most of the remaining fumarase. The cleaving enzyme derived from liver sources was evidently more labile during heating than the enzyme purified from yeast, since the latter could be heated at 60° for periods varying from 8 to 15 minutes. The resulting coagulum was rapidly cooled in a −5° ice bath, diluted to 1 ml. with 0.05 m potassium acetate buffer, pH 5.4, to give a protein concentration of 25 mg. per ml. This concentrated solution was heated in a water bath at 59° for 1.5 minutes and then rapidly cooled.

The coagulum was removed by centrifugation. The second heat step resulted in some loss of enzymatic activity but was retained because of its effectiveness in causing the destruction of most of the remaining fumarase. The cleaving enzyme derived from liver sources was evidently more labile during heating than the enzyme purified from yeast, since the latter could be heated at 60° for periods up to 15 minutes with less than 10 per cent loss of activity. Table I shows approximate yields and degrees of purification of the cleaving enzyme when carried through the procedures described. In the case of the liver-derived enzyme, a unit of enzyme activity is defined as the amount of enzyme which forms 0.01 μmole of AICAR in 30 minutes at 38° from 0.06 μmole of succino-AICAR in a volume of 0.43 ml. at pH 7.0.

The rate of formation of AICAR under these conditions is linear if less than 0.03 μmole of AICAR is synthesized.

**Isolation of Cleaving Enzyme from Yeast**—The purest preparations of cleaving enzyme obtained were prepared from bakers' yeast by a procedure, the initial steps of which were similar to those used by Carter and Cohen (4) in purifying adenylosuccinase. One kg. of dry bakers' yeast was suspended in 3 l. of 0.1 m NaHCO₃ and stirred at 37° for 5 hours. The mixture was frozen, thawed, and stirred at 37° for an additional hour. The autolysate was centrifuged in the Stock centrifuge at 5,300 × g for 30 minutes. To the supernatant fluid 625 gm. of ammonium sulfate were added slowly at room temperature. The mixture was then stirred for an additional 30 minutes and the insoluble material was collected by centrifugation as before. The precipitate was taken up in 400 ml. of distilled water at 2°, 80 gm. of ammonium sulfate were added slowly, and the mixture was stirred at 2° for 20 minutes. The precipitate was removed by centrifugation at 15,000 × g at 2° and discarded. Ammonium sulfate, 35 gm., was added to the supernatant solution while the temperature was brought to 25°. The precipitate was collected by centrifugation at this temperature and dissolved in 175 ml. of cold distilled water. The resulting solution contained appreciable amounts of fumarase as well as high activity for the cleaving of both succino-AICAR and adenylosuccinate. It was possible to store this preparation at −20° without loss of enzymatic activity. It is interesting to note that this solution had a much higher activity for the cleaving of succino-AICAR than did the most highly purified chicken liver fractions.

In order to remove fumarase and to accomplish further purification, the solution, prepared as described above, was heated at 60° for 10 to 15 minutes. The exact time of heating was predetermined by heating 0.1 ml. aliquots in small glass test tubes for periods varying from 8 to 15 minutes. The resulting coagulated fluid was rapidly cooled in an ice bath, diluted to 1 ml. with 0.1 m potassium phosphate buffer, pH 7.3, and assayed for fumarase and succino-AICAR cleaving activity. The period of heating which permitted retention of 90 per cent of the cleaving activity with complete inactivation of fumarase was then employed in treating the remainder of the fraction.

The cleaving enzyme was chromatographed on DEAE-cellulose (6) to obtain further purification. Eastman DEAE-cellulose was washed a number of times with water and then neutralized to pH 7.2. For large scale preparations a glass column with an inside diameter of 9 mm. was employed, the ion exchanger being loosely packed in the column to a height of 20 cm. The entire supernatant solution obtained by heating ammonium sulfate fractions from 1 kg. of dry yeast was dialyzed against 0.005 m potassium phosphate buffer at pH 7.2, and then poured onto the washed column. Elution of the column was accomplished by gradient elution, the upper vessel containing 0.15 m potassium phosphate, pH 5.9, and the mixing vessel containing 0.01 m potassium phosphate, pH 7.2. The cleaving enzyme was eluted after approximately 0.65 l. had passed through the column. Fractions containing significant cleaving activity were pooled and the pH adjusted to 7.2. This preparation was stable at −20° for a number of days, although some loss of activity occurred on dialysing or on thawing and refreezing the solution a number of times.

**Isolation of Cleaving Enzyme from N. crassa**—Mycelial extracts of N. crassa, strain 74 A, were found to contain considerable amounts of cleaving activity for both succino-AICAR and adenylosuccinic acid. The mold was grown for 3 days on Fries mini-

### Table I

**Purification of cleaving enzyme from liver**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein (mg)</th>
<th>Units of enzyme activity</th>
<th>Total units</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Extract</td>
<td>25,000</td>
<td>0.22</td>
<td>5450</td>
<td>100</td>
</tr>
<tr>
<td>II. 1st ethanol fractionation</td>
<td>3,000</td>
<td>1.70</td>
<td>5100</td>
<td>93</td>
</tr>
<tr>
<td>III. 1st heat step</td>
<td>1,580</td>
<td>2.75</td>
<td>4350</td>
<td>80</td>
</tr>
<tr>
<td>IV. Gel supernatant fluid</td>
<td>1,270</td>
<td>3.25</td>
<td>4130</td>
<td>76</td>
</tr>
<tr>
<td>V. 2nd ethanol fractionation</td>
<td>1,100</td>
<td>3.75</td>
<td>4130</td>
<td>76</td>
</tr>
<tr>
<td>VI. 2nd heat step</td>
<td>800</td>
<td>3.25</td>
<td>2600</td>
<td>43</td>
</tr>
</tbody>
</table>

3 All phosphate buffers mentioned in the text were made up by dissolving a weighed amount of KH₂PO₄ or NaH₂PO₄·H₂O in water, neutralizing to the desired pH with NaOH or KOH, and adding water to volume.

4 Purchased from Wilhelm Stock, Marburg, Germany.
nal medium, with 40 mg of adenine sulfate added per liter of culture in the case of the "F" mutants which require adenine for growth (7). The mycelia were filtered on a Buchner funnel, washed with water, and ground with clean sand in cold 0.05 M potassium phosphate buffer, pH 7.0. The mixture was allowed to stand for 1 hour at 2º. Centrifugation in a Servall centrifuge at 15,000 g, removed sand, intact mycelia, and insoluble fatty fractions and left a cloudy supernatant fluid which was used in the enzymatic tests.

*Chromatography—* Paper chromatography was employed in identifying the products of the cleavage of succino-AICAR. Succino-AICAR, formed from C4-aspartic acid, was incubated with 2 mg of purified cleaving enzyme from chicken liver or with 0.05 mg of highly purified cleaving enzyme from yeast for 15 to 30 minutes at 37º in a volume of 0.45 ml. After incubation the vessels were immersed in a boiling water bath for 1 minute, and placed in a clinical centrifuge for 5 minutes. The clear supernatant solution was drawn off and evaporated to 0.1 ml. over P2O5 in a vacuum. The residual solution was chromatographed on Whatman No. 1 paper with the descending technique. A solvent consisting of 4 N formic acid saturated with n-butanol was found to be suitable for identification of the dicarboxylic acid formed initially when succino-AICAR is converted to AICAR. This solvent separates succinic, malic, pyruvic, maleic, and oxaloacetic acids from fumaric acid. The position of the dicarboxylic acids on the chromatograms was ascertained by spraying with an ethanolic solution of bromoresol green and by determination of radioactivity. The distribution of radioactivity was determined by ruling the paper into squares and by counting each square over the window of a Geiger counter. The nucleotides remain within 2 cm. of the origin and thus do not interfere with the determination of radioactivity in the dicarboxylic acid spots.

**Assay Procedures: Enzymatic Activities**—In the case of the enzymes obtained from yeast and Neurospora, cleaving activity was measured by incubating aliquots of enzyme solutions for 3 minutes at 38º with 0.061 μmole of succino-AICAR and 4.5 μmoles of potassium phosphate buffer, pH 7.2, in a final volume of 0.65 ml. The reaction was stopped by addition of 0.15 ml of 30 per cent trichloroacetic acid and a 0.4 ml aliquot was withdrawn for determination of AICAR by the modified Bratton and Marshall assay (5). A unit of enzymatic activity was defined as that amount of enzyme which caused a change in absorbance of 1.0 in 1 minute. The molecular extinction coefficient of adenylosuccinate was taken as 13,600 at 280 mμ (4).

Fumarase—Fumarase activity was determined spectrophotometrically. Each Beckman cuvette contained initially 2.48 μmoles of sodium fumarate, 270 μmoles of potassium phosphate buffer, pH 7.3, and protein in a final volume of 3.0 ml. The absorbance at 250 mμ was determined as a function of time. A control vessel contained the same amount of protein as the reaction vessel, but lacked sodium fumarate. The molecular extinction coefficient of fumaric acid at 250 mμ is 1.430 (8). Under the conditions described, the absorbance of the reaction mixture before starting the reaction was 1.20. Since at equilibrium about 80 per cent of the fumarate was converted to maleate (8, 9), the absorbance at equilibrium was 0.240, maleate having virtually no absorption at the wave length employed.

**Protein**—Protein concentrations were determined spectrophotometrically by measuring the ultraviolet absorption at 280 mμ in the case of the liver preparations. It was assumed that 1 mg of protein per ml gave an absorbance of 1.6 in a light path of 1 cm. (10). In estimating protein concentrations of extracts of microorganisms the Lowry method was employed (11). Bovine albumin was used as a standard in the latter assay.

**RESULTS**

A consideration of the structures of succino-AICAR and Adenylosuccinase reveals that the 4-carbon chain derived from aspartic acid has been cleaved in the conversion of succino-AICAR to AICAR. In earlier experiments radioactive succino-AICAR derived enzymatically from aspartic acid-4-C14 yielded on cleavage, two radioactive, acid-reacting spots corresponding to fumaric and maleic acids. In the presence of crude cleaving enzyme in chicken liver extract, 3 to 4 times more maleate than fumarate was formed, as judged by the radioactivity present in the 2 spots. The ratio of maleate to fumarate observed in this case is in agreement with the values for the malate-fumarate equilibrium in the presence of fumarase reported by Alberty et al. (8). When the cleaving enzyme had been partially purified it became possible to positively identify the dicarboxylic acid which was formed by the action of the cleaving enzyme on succino-AICAR. Table II illustrates the results of a chromatographic analysis of incubation mixtures after cleaving of succino-AICAR with enzyme at different stages of purification.

As may be seen from the results of Vessel 3, Table II, fumarate was the exclusive dicarboxylic acid product of the reaction when short incubations (15 minutes) with purified cleaving enzyme were employed. A slight radioactive spot corresponding to maleate could be detected when longer incubations were used (Vessel 2). This finding would indicate that very small residual amounts of fumarase are still present in the purified enzyme. In Vessel 4, 10 μmoles of unlabeled fumarate were added to the incubation mixture before chromatography. Only one ultraviolet, acid-reacting spot was detected, which coincided exactly with the single radioactive spot found in the region of the chromatogram corresponding to fumaric acid. When the most purified fraction from yeast was employed in a similar experiment no maleate could be detected by chromatography even after an incubation period of 2 hours. This preparation was thus free from traces of fumarase. These results establish that fumaric acid is the dicarboxylic acid formed by the action of the cleaving enzyme on succino-AICAR.

**Evidence for Identity of Cleaving Enzyme and Adenylosuccinase**—The circumstantial evidence favoring the probability that the activities for the cleaving of the succino-AICAR and adenylosuccinase-
0.05 M potassium acetate buffer, pH 5.4. Chromatography was carried out as described under "Methods."

Each incubation vessel contained 0.10 pmole of succino-AICAR and the indicated enzyme fraction which had been dissolved in 0.05 M potassium acetate buffer, pH 7.4. Chromatography was carried out as described under "Methods."

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Addition to vessel</th>
<th>Incubation time (min.)</th>
<th>Acid reaction with bromocresol green</th>
<th>Radioactivity in acid reacting area (c.p.m.)</th>
<th>Ident. of products of reaction</th>
<th>RF</th>
</tr>
</thead>
</table>
| 1      | 30 to 40% ethanol fraction of chicken liver extract | 15                    | + 0.838                          | 557                              | + |<|+
| 2      | Partially purified enzyme* | 15                    | + 0.813                          | 314                              | + |<|+
| 3      | Partially purified enzyme | 5                     | - 0.426                          | 16                               | - |<|-
| 4      | Partially purified enzyme plus unlabeled fumarate (6 μmoles) | 15                    | + 0.806                          | 586                              | + |<|+

| Fumarate marker | Malate marker | + 0.806 | + 0.426 |

* Succino-AICAR cleaving enzyme purified from chicken liver through the second heat step.
† Radioactivity measurements were made at the Rf of malic acid even in the absence of a visible acid reaction in this vicinity.

Concurrence of Cleaving Activity with Adenylosuccinase during Purification from Yeast—As shown in Table III, the ratio of adenylosuccinase activity to succino-AICAR cleaving activity of the yeast enzyme remained constant within the limit of error of the methods. Although evidence of this type is most significant only when performed on a highly purified enzyme, the present findings are indicative, however, of the identity or nonidentity of these enzyme activities with the use of an enzyme preparation from N. crassa (Strain 74A). Mycelial extracts of the wild type organism contain both activities for cleaving succino-AICAR and adenylosuccinic acid. When analysis was made in this laboratory for the cleaving of succino-AICAR in the "F" mutants (F-5, F-7, F-12) it was found that this activity was likewise absent. It is thus seen that this mutation is responsible for the loss of both enzymatic reactions and that in all probable

| TABLE II
| Identification of dicarboxylic acid liberated in cleavage of succino-AICAR—C14

Each incubation vessel contained 0.10 pmole of succino-AICAR and the indicated enzyme fraction which had been dissolved in 0.05 M potassium acetate buffer, pH 7.4. Chromatography was carried out as described under "Methods."

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| 3      | Partially purified enzyme | 5                     | - 0.426                          | 16                               | - |<|-
| 4      | Partially purified enzyme plus unlabeled fumarate (6 μmoles) | 15                    | + 0.806                          | 586                              | + |<|+

| Fumarate marker | Malate marker | + 0.806 | + 0.426 |

| TABLE III
| Purification of adenylosuccinase and succino-AICAR cleaving enzyme from yeast

Each vessel contained 90 mmoles of succino-AICAR and 0.8 mg of cleaving enzyme purified from chicken liver in a final volume of 0.80 ml. All vessels were incubated at 37° for 20 minutes. The reaction was terminated by addition of 0.15 ml of 30 per cent trichloroacetic acid and denatured protein was removed by centrifugation. AICAR was determined in a 0.40 ml aliquot.

| TABLE IV
| Inhibition of conversion of succino-AICAR to AICAR by adenylosuccinic acid

Each vessel contained 90 mmoles of succino-AICAR and 0.8 mg of cleaving enzyme purified from chicken liver in a final volume of 0.80 ml. All vessels were incubated at 37° for 20 minutes. The reaction was terminated by addition of 0.15 ml of 30 per cent trichloroacetic acid and denatured protein was removed by centrifugation. AICAR was determined in a 0.40 ml aliquot.

| TABLE V
| Identification of dicarboxylic acid liberated in cleavage of succino-AICAR—C14

Each incubation vessel contained 0.10 pmole of succino-AICAR and the indicated enzyme fraction which had been dissolved in 0.05 M potassium acetate buffer, pH 7.4. Chromatography was carried out as described under "Methods."

<table>
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<th>Addition to vessel</th>
<th>Incubation time (min.)</th>
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| Fumarate marker | Malate marker | + 0.806 | + 0.426 |

* Succino-AICAR cleaving enzyme purified from chicken liver through the second heat step.
† Radioactivity measurements were made at the Rf of malic acid even in the absence of a visible acid reaction in this vicinity.
Effect of Ionic Strength on Succino-AICAR Cleaving Enzyme and Adenylosuccinase—Under carefully controlled conditions of pH, both enzymatic activities were influenced appreciably by the ionic strength of the incubation medium. This effect was produced by monovalent and divalent ions, including ions of the following salts: KCl, LiCl, NaCl, K2SO4, NH4Cl, NaH2PO4 (pH 7.2), MgCl2 (pH 7.2, sodium and potassium free), imidazole hydrochloride (pH 7.0), tris(hydroxymethyl)aminomethane (pH 7.2). The effect of KCl solutions on the enzymatic activities is illustrated in Fig. 1, the optimal ionic strength for both enzymatic activities being about 0.063 M per liter. On preincubation of 0.04 unit of the purest fraction of the succino-AICAR cleaving enzyme in 0.003 M sodium phosphate buffer, pH 7.3, at 37° for 10 minutes, most of the enzymatic activity was lost if assayed without adding any additional salts to the incubation mixture. If, however, the preincubation were carried out in the presence of 0.063 M sodium phosphate buffer, pH 7.2, most of the activity was preserved. Addition of sodium phosphate buffer after preincubation at low ionic strength restored up to 50 per cent of the activity. This finding may indicate that the effect of salts is to maintain the enzyme in an active form.

Characteristics of Succino-AICAR Cleaving Enzyme: pH Optimum—The optimal pH for the cleaving of succino-AICAR in the presence of 0.01 M sodium phosphate buffer was 7.3. At pH 4 and 9 with other buffers of equivalent ionic strength, no enzymatic activity was observed under the conditions described in Fig. 2. The optimal enzyme activity is in the neighborhood of pH 7.8 when a 0.01 M Tris buffer is used. It has been shown by Carter and Cohen (4) that the pH optimum of adenylosuccinase is in this same range.

Determination of Michaelis Constant of Succino-AICAR—The method of Lineweaver and Burk (13) was used to determine the Michaelis constant of succino-AICAR at various ionic strengths and enzyme concentrations. When the concentration of succino-AICAR was varied from 0.08 mM to 0.90 mM in the presence of 0.015 M sodium phosphate buffer, pH 7.3, the K_m was found to be 1.9 × 10^-4 M as reported in a previous publication (1). This determination was made with a relatively crude enzyme preparation. In later experiments where the most highly purified enzyme preparation was used somewhat smaller values of K_m were obtained. For example when the concentration of phosphate buffer in the incubation medium was 0.04 M, a value of 1.1 × 10^-4 M was observed.

Table V

<table>
<thead>
<tr>
<th>AICAR</th>
<th>Succino-AICAR</th>
<th>Fumarate</th>
<th>K_m × 10^4 moles per l.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.28</td>
<td>0.87</td>
<td>6.21</td>
<td>2.0</td>
</tr>
<tr>
<td>0.34</td>
<td>0.71</td>
<td>4.49</td>
<td>2.2</td>
</tr>
<tr>
<td>0.91</td>
<td>2.06</td>
<td>5.63</td>
<td>2.5</td>
</tr>
<tr>
<td>1.23</td>
<td>1.86</td>
<td>2.88</td>
<td>1.9</td>
</tr>
<tr>
<td>0.60</td>
<td>0.97</td>
<td>4.70</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Average ........................................ 2.3 ± 0.3
Equilibrium between Succino-AICAR, AICAR, and Fumaric Acid—Equilibrium between the three substrates was accomplished by incubating varying amounts of fumaric acid and AICAR or fumaric acid and succino-AICAR or all three with 0.5 unit of the most purified cleaving enzyme and 10 μmoles of potassium phosphate buffer, pH 7.2, at 37° for 180 minutes. The vessels were heated after incubation and succino-AICAR and AICAR were determined. Values for the equilibrium constant obtained with highly purified cleaving enzyme are shown in Table V. The value for the equilibrium constant published in a preliminary report of this work was obtained with preparations of cleaving enzyme which were only 35-fold pure and thus may have contained minute amounts of fumarase, the effect of which would only be noticeable after several hours of incubation. No fumarase activity was detected in the purest preparations from yeast even after 4 hours of incubation under the conditions described under "Methods" for determination of fumarase.

DISCUSSION

Partridge and Giles have recently demonstrated that 2 revertants of an "F" mutant regain the same percentage of the lost enzymatic activity whether assayed for adenylosuccinase or succino-AICAR cleaving enzyme. These facts may indicate that the loss of both activities is the result of a single genetic event. The indication that a single mutation exerts the same effect on 2 enzymatic activities makes it probable that only one enzyme is involved. The other evidence presented, including inhibition of the cleaving of succino-AICAR by adenylosuccinate and 6-(1,2-dicarboxyethylmercapto)-9-β-d-ribofuranosylpurine 5'-phosphate and coincidence of the 2 activities after 170-fold purification, would tend to substantiate this view. A consideration of the structures of adenylosuccinic acid and succino-AICAR does not make this result seem too surprising since both ribonucleotides possess the imidazole ring, as well as the succino moiety. Despite the similarity of the cleavage of argininosuccinic acid to the cleavage of adenylosuccinate and succino-AICAR, no argininosuccinate activity was present in the purified succino-AICAR cleaving enzyme.

Gots and Gollub (14) working with mutants of E. coli have provided similar evidence for the identity of adenylosuccinase and the succino-AICAR cleaving enzyme.

SUMMARY

An enzyme which converts N-(5-amino-1-ribosyl-4-imidazolylcarbonyl)-L-aspartic acid 5'-phosphate (succino-AICAR) to 5-amino-1-ribosyl-4-imidazolecarboxamide 5'-phosphate (AICAR) and fumaric acid has been purified 170-fold from bakers' yeast. The ratio of activity of this enzyme to adenylosuccinase activity remains constant during purification. Several microbial mutants lacking adenylosuccinase have now been demonstrated to lack also the ability to carry out the cleavage of succino-AICAR. Behavior of the enzyme toward substrates and inhibitors lends further support to the view that both activities reside in a single enzyme. The equilibrium constant for the cleavage of succino-AICAR is 2.3 ± 0.3 x 10^-4 moles per l. The K_m of the enzyme for succino-AICAR is 1.1 x 10^-4 moles per l in the presence of 0.04 M phosphate buffer.

REFERENCES

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