Lysine Biosynthesis in *Saccharomyces*

CONVERSION OF α -AMINOADIPATE INTO α -AMINOADIPIC δ -SEMIALDEHYDE

BY ASRU K. SINHA AND J. K. BHATTACHARJEE

Department of Microbiology, University of Miami, Oxford, Ohio 45056, U.S.A.

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The reduction of α -aminoadipate to α -aminoadipic δ -semialdehyde by a cell-free extract of *Saccharomyces* is shown to be a three-step process. First the amino acid reacts with ATP to form an adenylyl derivative. Then the adenylyl derivative of α -aminoadipate is reduced in the presence of NADPH. In the third step the reduced adenylyl derivative of the amino acid is cleaved to form α -aminoadipic δ -semialdehyde. The presence of Mg^{2+} is necessary for the first and second steps. The third step does not need any cofactors. The product of the first step was isolated by chromatography after incubating the cell-free extract of *Saccharomyces* with α -aminoadipate, ATP and Mg^{2+} . The isolated product was identified as an adenylyl derivative of α -aminoadipate and could be converted into α -aminoadipic δ -semialdehyde under the stated experimental conditions. The product of the second step was too unstable to be identified.

The reduction of α -aminoadipate to α -aminoadipic δ -semialdehyde (referred to below as aminoadipate and aminoadipic semialdehyde respectively) is thought to be an obligatory step for lysine biosynthesis in *Saccharomyces* and other fungi (Sagisaka & Shimura, 1959; Jones & Broquist, 1965).

Although the conversion of aminoadipate into its semialdehyde derivative has been postulated to be a multi-step process (Scheme 1; Sagisaka & Shimura, 1962), this has not yet been demonstrated. In the present paper we show that this conversion is indeed a multi-step reaction, and attempts have also been made to isolate the postulated intermediates involved in the reduction process.

MATERIALS AND METHODS

Organisms. Wild-type strain (WL-1) and a lysine auxotroph ly_3 (no. AB9) of *Saccharomyces* were obtained from Professor C. C. Lindegren, Southern Illinois University, Carbondale, Ill., U.S.A. Strain WL-1 was grown in liquid medium containing 'Bacto Yeast Nitrogen Base (without amino acid)' (0.67%) and glucose (1%) in distilled water. The mutant ly_3 was grown in the same medium, supplemented with L-lysine (10 mg/100 ml). The pH of the media was 4.5.

Preparation of yeast extract. Cells were grown in Erlenmeyer flasks (500 ml) containing liquid medium (150 ml) at 30°C for 36 h by using a Metabolyte (model G77) water-bath shaker (200 rev./min), and harvested by centrifugation at 0°C. Except where buffers of different pH values were used, the extracts were obtained by disrupting the cells suspended in 0.01 M-tris-HCl buffer, pH 8.0, in a precooled 50 ml homogenizing flask containing

chilled glass beads (0.45–0.50 mm). The cell mass was disrupted in a Bronwill MSK homogenizer for 35 s at 3450 rev./min. During disruption the temperature was controlled below 5°C by using liquid CO₂. The disrupted mass was centrifuged at 15000 rev./min (27000g) for 30 min at 0°C in a Sorvall centrifuge. The supernatant extract was used as such or dialysed against 0.01 M-tris-HCl buffer, pH 8.0, for 12 h at 8°C before use.

Assay of aminoadipic semialdehyde formation by cell-free extracts. Formation of aminoadipic semialdehyde by the cell-free extracts was determined by the procedure of Sagisaka & Shimura (1959). Unless otherwise stated the incubation mixture contained 2.5 μ mol of DL-aminoadipic acid, 3.0 μ mol of ATP, 2.0 μ mol of $MgCl_2 \cdot 6H_2O$, 80 μ g of GSH, 0.5 μ mol of NADPH, 200 μ mol of tris-HCl buffer, pH 8.0, and cell extract (5 mg of protein) to a final volume of 1.0 ml. After incubation at 30°C for 90 min the formation of aminoadipic semialdehyde was determined with *p*-dimethylaminobenzaldehyde reagent at pH 7.0 by measuring the extinction at 460 nm. Where EDTA was added to the reaction mixture the pH of the EDTA solution was adjusted to the pH of the incubation mixture.

Complementation *in vitro* of aminoadipic semialdehyde-forming activities of extracts of strains WL-1 and ly_3 was studied by the method described by Sinha & Bhattacharjee (1970).

Hydroxamate assay for the activation of aminoadipate. Activation of aminoadipate by extracts of strains WL-1 and ly_3 was determined by the method of Lipmann & Tuttle (1945). The incubation mixture contained 2.5 μ mol of DL-aminoadipic acid, 3 μ mol of ATP, 2.0 μ mol of $MgCl_2 \cdot 6H_2O$, 200 μ mol of K_2HPO_4 - KH_2PO_4 buffer, pH 7.2, and extract equivalent to 2–5 mg of protein in a total volume of 1.0 ml. After incubation at 30°C for 20 min, 0.25 ml of each of NH_2OH (28% neutralized

solution) and 0.1M-acetic acid-sodium acetate buffer, pH 5.4, were added. After 10 min 0.5 ml of each of 3.0M-HCl, 10% (w/v) trichloroacetic acid and 5% (w/v) FeCl₃ solution in 0.1M-HCl were added successively. The precipitated proteins were removed by centrifugation and the extinction at 540 nm was measured in a Hitachi-Perkin-Elmer spectrophotometer.

Exchange of [³²P]PP_i. PP_i exchange studies during the reaction between ATP and aminoadipate were done by the procedure of Hoagland, Keller & Zamecnik (1956). The incubation mixture contained 2.5 μmol of DL-aminoadipic acid, 3.0 μmol of ATP, 2.0 μmol of MgCl₂·6H₂O, 12 μmol of KF, 1 μmol of [³²P]PP_i (1.4 × 10⁵ c.p.m.), 200 μmol of tris-HCl buffer, pH 7.5, and extract to a final volume of 1.2 ml. After incubation at 30°C for 20 min, the reaction was stopped by the addition of 1 ml of 10% (w/v) trichloroacetic acid. PP_i and ATP were separated by using 150 mg of activated charcoal and the percentage exchange was calculated by the procedure of Crane & Lipmann (1953). Radioactivity was determined with a Beckman Scintillation Spectrometer (model LS-100, counting efficiency approx. 60%).

Isolation of products of the reaction in vitro. The reaction products formed by the cell-free extracts of strains WL-1 and ly₅ in the presence and in the absence of NADPH were isolated in tracer experiments with amino[6-¹⁴C]adipate. To the reaction mixtures described above approx. 5 μCi of the radioactive amino acid was added. After the incubation the reaction mixtures were adsorbed on a Dowex 1 (formate form) column (20 cm × 0.9 cm) at 4°C. The column was eluted twice with 10 ml of chilled water and subsequently with 10 ml each of cold 0.5M, 1.0M, 1.5M, 2.0M, 2.5M, 3.0M, 3.5M, 4.0M, 5.0M and 6.0M-formic acid. Portions (0.5 ml) of each fraction, including the water washings, were evaporated to dryness and the radioactivity was measured in a toluene-ethanol solvent system (Kobayashi, 1966).

Fractions containing radioactivity were freeze-dried and used for further experiments as necessary. The adenylyl moiety in the radioactive product was identified by the method of Gerlach & Doring (1955).

Spectrophotometry. In the assay for the conversion of aminoadipate into aminoadipic semialdehyde the oxidation of NADPH was also measured. A typical incubation mixture contained, in a total volume of 3.5 ml, 8.7 μmol of DL-aminoadipic acid, 11 μmol of ATP, 7.0 μmol of MgCl₂·6H₂O, 8 μmol of NADPH, 350 μmol of tris-HCl buffer, pH 8.0, and 5 mg of the cell-free extract. At different times portions of the reaction mixture were taken for the determination of the amounts of aminoadipic semialdehyde formed and NADPH oxidized. The NADPH was measured spectrophotometrically at 340 nm in a Hitachi-Perkin-Elmer spectrophotometer. The ε of NADPH was taken to be 6.22 × 10³ l. mol⁻¹. cm⁻¹.

Chromatography. Amino[¹⁴C]adipate and its semialdehyde derivative were identified by paper chromatography with the solvent systems described by Fjellstedt & Ogur (1970) with the help of carrier compounds. After chromatography the papers were cut into strips and scanned in a radiochromatogram scanner to locate the radioactive compounds.

Protein determination. Protein content of the cell-free extracts was determined by the biuret method (Gornall, Bardawill & David, 1949).

Chemicals. Aminoadipic semialdehyde used in this study was prepared enzymically and purified by chromatography as described by Sagisaka & Shimura (1959). NADPH and DL-aminoadipic acid were products of the Sigma Chemical Co., St Louis, Mo., U.S.A.; DL-α-amino[6-¹⁴C]adipic acid was bought from the International Chemical and Nuclear Corp., Irvine, Calif., U.S.A. Bacto Yeast Nitrogen Base (without amino acid) was the product of Difco Laboratories, Detroit, Mich., U.S.A.

RESULTS

Activation and reduction of aminoadipate by cell-free extracts of strains ly₅ and WL-1 and the role of Mg²⁺ in these steps. It has been reported that the *Saccharomyces* lysine auxotroph ly₅ cannot utilize aminoadipate for lysine biosynthesis. This was attributed to the inability of the mutant to convert aminoadipate into aminoadipic semialdehyde (Jones & Broquist, 1965; Sinha & Bhattacharjee, 1970).

From the postulated mechanism (Scheme 1) it is obvious that the malfunctioning of any of the three steps could prevent the extract of strain ly₅ from forming aminoadipic semialdehyde from aminoadipate. The following experiments were done to test whether the residual activities of any

Scheme 1. Proposed mechanism for the reduction of aminoadipate to aminoadipic semialdehyde.

one or more steps related to the reduction of amino-adipate were present in the cell-free extract of strain *ly_s*. The activation of aminoadipate (step 1 in Scheme 1) by the cell-free extract of strain *ly_s* was determined by hydroxamate formation. The cell-free extract was incubated with aminoadipate, ATP and Mg^{2+} and tested with neutralized hydroxylamine and ferric chloride reagent (Lipmann & Tuttle, 1945); the hydroxamate formation was compared with that obtained with extract of strain WL-1 under identical experimental conditions. The activity of step 1 was present in extracts of both strains *ly_s* and WL-1 (Fig. 1). The reaction was found to require Mg^{2+} . The activity was, however, relatively low in both cases and the reaction seemed to cease within 15 min.

The activation of aminoadipate in the presence of ATP and Mg^{2+} by the cell-free extracts of the organisms was also studied by the $[^{32}P]PP_i$ exchange reaction (Hoagland *et al.* 1956). The percentage of $[^{32}P]PP_i$ exchange in the extract of strain *ly_s* was similar to that obtained with extract of strain WL-1 (Table 1). The PP_i exchange reaction was also found to be dependent on Mg^{2+} . The above results demonstrate that the activity of step 1 (Scheme 1) is present in the mutant *ly_s*.

In the cell-free extract of strain WL-1 the oxidation of NADPH occurred concomitantly with the formation of aminoadipic semialdehyde from aminoadipate in the presence of ATP and Mg^{2+} (Fig. 2). Since the oxidation of NADPH related to the reduction of aminoadipate by cell-free extract of *Saccharomyces* is postulated to occur in step 2 (Scheme 1), the rate of oxidation of

NADPH by cell-free extract of strain *ly_s* was measured in an identical reaction mixture to determine the activity of step 2 in the mutant. The extract of strain *ly_s* oxidized NADPH without the formation of any aminoadipic semialdehyde (Fig. 2). Omission of aminoadipate or ATP or Mg^{2+} resulted in very little oxidation of NADPH by the extract of strain *ly_s*. Addition of EDTA to the reaction mixtures (20 μ mol/ml) drastically inhibited the oxidation of NADPH, and in the extract of strain WL-1 no aminoadipic semialdehyde was formed. The inhibitory effect of EDTA was partly overcome (approx. 48%) by increasing the amount of Mg^{2+} in the reaction mixture (3.5 ml) to 30 μ mol.

Table 1. *Pyrophosphate exchange reaction by the cell-free extracts of Saccharomyces strains WL-1 and ly_s*

The reaction mixture contained 2.5 μ mol of DL- α -aminoadipic acid, 3.0 μ mol of ATP, 2.0 μ mol of $MgCl_2 \cdot 6H_2O$, 12 μ mol of KF, 1 μ mol of $[^{32}P]PP_i$ (1.4×10^5 c.p.m.), 200 μ mol of tris-HCl buffer, pH 7.5, and 5 mg of cell-free extract in a total volume of 1.2 ml. The details of the experiment are given in the Materials and Methods section.

Cell-free extract	Omission from the reaction mixture	% exchange
Strain WL-1	None	40.2
Strain WL-1	Aminoadipate	10.4
Strain WL-1	Mg^{2+}	14.6
Strain <i>ly_s</i>	None	43.4
Strain <i>ly_s</i>	Aminoadipate	12.8
Strain <i>ly_s</i>	Mg^{2+}	19.0

Fig. 1. Activation of aminoadipate by the cell-free extract of strain WL-1 in the presence (□) and in the absence (Δ) of Mg^{2+} . Formation of hydroxamate was measured by the procedure of Lipmann & Tuttle (1945). The activation of aminoadipate by the cell-free extract of strain *ly_s* (●) was studied under identical conditions. For details see the Materials and Methods section.

Fig. 2. Oxidation of NADPH by cell-free extracts of strain WL-1 (●) and strain *ly_s* (○) and the effect of EDTA (20 μ mol/ml of the reaction mixture) on the reactions. Formation of aminoadipic semialdehyde in extracts of strain WL-1 (▲) and strain *ly_s* (Δ) was assayed simultaneously with the measurement of oxidation of NADPH, as described in the text.

The above results showed that the mutant *ly_s* does have the activities of the steps 1 and 2, and the failure of this mutant to convert aminoadipate into aminoadipic semialdehyde can be attributed to the loss of the cleavage reaction (step 3 in Scheme 1) from the enzyme system.

Formation of aminoadipic semialdehyde by complementation in vitro of cell-free extract of strain *ly_s* and EDTA-treated extract of strain of WL-1. The loss of the cleavage step (step 3 in Scheme 1) from the enzyme system involved in the reduction of aminoadipate in strain *ly_s* was demonstrated *in vitro* by complementation between the extract of strain *ly_s* and EDTA-treated extract of strain WL-1. The cell-free extract of strain *ly_s* was incubated with aminoadipate, ATP, Mg^{2+} and NADPH. After 1 h of incubation at 30°C, freshly prepared EDTA-treated extract of strain of WL-1 was added to the reaction mixture and the incubation was continued for another 1 h. The formation of semialdehyde was then determined with *p*-dimethylaminobenzaldehyde reagent. Formation of aminoadipic semialdehyde by extract of strain *ly_s* complemented with EDTA-treated extract of strain WL-1 was demonstrated in the reaction system (Table 2). Since the cell-free extract of the mutant and the EDTA-treated extract of strain WL-1 individually did not form any aminoadipic semialdehyde under the above reaction conditions, in the complementation process the EDTA-treated extract of strain WL-1 must have provided the source of catalyst for the cleavage reaction (step 3 in Scheme 1), missing from the mutant *ly_s*. Further, whereas the rate of oxidation of NADPH by the cell-free extracts of strains *ly_s* and WL-1 were comparable, the formation of aminoadipic semialdehyde in the

complementation reaction was about 50% of the amount formed with extract of strain WL-1 alone. The discrepancy might possibly arise from the breakdown of the intermediate necessary for the aminoadipic semialdehyde formation in the absence of a catalytic source for the cleavage reaction (step 3 in Scheme 1).

Isolation and identification of products of the reaction in vitro. The products of aminoadipate metabolism by the cell-free extracts of the organisms in the presence and in the absence of NADPH were isolated by tracer experiments in which extracts were incubated with amino[6- ^{14}C]-adipate, ATP and Mg^{2+} , with or without NADPH. Where the formation of aminoadipic semialdehyde was studied, GSH was also included in the reaction mixture. After incubation at 30°C for the stated time, the reaction mixture was adsorbed on a Dowex 1 (formate form) column at 4°C. The adsorbed materials were eluted from the column with water and different concentrations of formic acid as described in the Materials and Methods section. Elution patterns of reaction mixtures are shown in Table 3.

When the complete reaction mixture incubated in the presence of extract of strain WL-1 was fractionated on the ion-exchanger as described above, radioactive aminoadipic semialdehyde, which was identified by reaction with *p*-dimethylaminobenzaldehyde, appeared in the first washing with water. Unchanged aminoadipate was eluted with 2.0M- and 2.5M-formic acid (Table 3). A small radioactive peak was also noted in the 1.0M-formic acid fraction. No attempts were made to identify this material, but it might be the anhydride form of aminoadipic semialdehyde (Sagisaka & Shimura, 1959).

Table 2. *Formation of aminoadipic semialdehyde by complementation in vitro of the cell-free extracts of Saccharomyces strains WL-1 and ly_s*

The extract of strain *ly_s* (5 mg) was incubated in reaction mixture (total volume 1.0 ml) containing 2.5 μ mol of DL- α -aminoadipic acid, 3.0 μ mol of ATP, 2.0 μ mol of $MgCl_2 \cdot 6H_2O$, 80 μ g of GSH, 0.5 μ mol of NADPH and 200 μ mol of tris-HCl buffer, pH 8.0 for 1 h at 30°C. After 1 h, 1.0 ml of EDTA-treated extract of strain WL-1 (5 mg of protein) was added to the mixture. The final concentration of EDTA in the incubation mixture was 20 μ mol/ml. The incubation was continued for another 1 h at 30°C and the formation of aminoadipic semialdehyde was then assayed with *p*-dimethylaminobenzaldehyde reagent. Aminoadipic semialdehyde-forming activity of the EDTA-treated cell-free extract of strain WL-1 was measured in the identical reaction mixture with the same final concentration of EDTA (20 μ mol/ml).

Cell-free extract	Addition for second incubation	Aminoadipic semialdehyde formed (μ mol)
Strain WL-1	None	0.31
Strain <i>ly_s</i>	Strain WL-1 (EDTA-treated)	0.15
Strain <i>ly_s</i> (heat-inactivated)	Strain WL-1 (EDTA-treated)	0.00
Strain WL-1 (EDTA-treated)	None	0.00
Strain <i>ly_s</i>	None	0.00

Table 3. Fractionation on a Dowex 1 (formate form) column of the products formed in vitro from amino[6- 14 C]adipate in the presence of extract of *Saccharomyces strains WL-1 and ly₅ and different cofactors*

The complete reaction mixture contained, in a total volume of 1.0 ml, 2.5 μ mol of DL-aminoadipic acid (containing 5 μ Ci of amino[6- 14 C]adipic acid), 3.0 μ mol of ATP, 2.0 μ mol of $MgCl_2 \cdot 6H_2O$, 80 μ g of GSH, 0.5 μ mol of NADPH, 200 μ mol of tri-HCl buffer, pH 8.0, and cell-free extract (5 mg of protein) of either strain WL-1 or ly₅. This reaction mixture is defined as the 'complete' mixture in the Table. Where the activation of aminoadipate by the cell-free extracts was studied, the reaction mixture contained 2.5 μ mol of DL-aminoadipic acid (containing 5 μ Ci of amino[6- 14 C]adipic acid), 3.0 μ mol of ATP, 2.0 μ mol of $MgCl_2 \cdot 6H_2O$, 200 μ mol of K_2HPO_4 -KH₂PO₄ buffer, pH 7.2, and 5 mg of cell-free extract in a total volume of 1.0 ml. This reaction mixture is defined as 'without NADPH' type of mixture in the Table. Both reaction mixtures were incubated at 30°C, the former for 90 min and the latter for 20 min. For details see the text.

Cell-free extract	Type of mixture	Eluent	Water (first)	Water (second)	Radioactivity eluted (c.p.m./ml)											
					0.5 M-		1.0 M-		1.5 M-		2.0 M-		3.0 M-		4.0 M-	
					Formic acid	Formic acid	Formic acid	Formic acid	Formic acid	Formic acid	Formic acid	Formic acid	Formic acid	Formic acid	Formic acid	Formic acid
Strain WL-1	Complete	15047	1023	328	540	4441	1238	293420	80500	9290	1753	5320	2142	601	320	590
Strain WL-1	Without NADPH	267	135	128	432	267	432	419815	62600	6442	1432	771	325	125	680	420
Strain ly ₅	Complete	160	155	503	721	650	679	382135	53320	3251	2142	4140	1386	672	420	290
Strain ly ₅	Without NADPH	282	190	240	330	412	820	393374	65994	5972	1225	772	248	290	290	290
Strain ly ₅ (heated)	Complete	190	240	330	412	820	393374	65994	5972	1225	772	248	290	290	290	290

To isolate the reaction products of the activation of aminoadipate, the amino acid containing amino-[6- 14 C]adipate was incubated with ATP, Mg^{2+} and the cell-free extract of strain WL-1. After incubation for 20 min at 30°C the reaction mixture was fractionated on a Dowex 1 (formate form) column as described above. A new radioactive material, which was absent from the control experiment with heat-inactivated extract of strain WL-1, was eluted by 3.5 M-formic acid ('without NADPH' in Table 3). The same results were obtained when the cell-free extract of strain WL-1 was replaced by that of strain ly₅ in an identical reaction mixture. The material in the 3.5 M-formic acid fraction contained an adenylyl group as shown by the method of Gerlach & Doring (1955). The material (0.9 mg) from the 3.5 M-formic acid fraction was hydrolysed by heating at 100°C for 18 h with 6.0 M-hydrochloric acid (1.0 ml) in a sealed tube. The presence of radioactive aminoadipate in the hydrolysate was shown by paper chromatography after the removal of hydrochloric acid under vacuum. To test whether this material was an intermediate in the formation of aminoadipic semialdehyde, it was incubated with the cell-free extract of strain WL-1, Mg^{2+} and NADPH. Not only was some of the material converted into aminoadipic semialdehyde, but a substantial portion of it was converted into aminoadipate (Table 4). The formation of radioactive aminoadipic semialdehyde and aminoadipate was initially deduced from their elution pattern from the Dowex 1 (formate form) column (Table 3) and later confirmed by paper chromatography of the eluted materials with the solvent systems described by Fjellstedt & Ogor (1970). An unidentified material was eluted from the Dowex 1 column by 1.0 M-formic acid. The radioactive aminoadipic semialdehyde could not have arisen from radioactive aminoadipate because the incubation system did not contain any ATP, which is necessary for this conversion (Sagisaka & Shimura, 1959). No other radioactive peaks were found to be present in the elution profile of the experiment with amino[6- 14 C]adipate, ATP, Mg^{2+} and cell-free extracts of strains ly₅ and WL-1 in the absence of NADPH (Table 3). Whereas as much as 12% of the aminoadipate was converted into aminoadipic semialdehyde by the cell-free extract in the presence of all the necessary cofactors, less than 1.0% was converted when NADPH was omitted from the incubation mixture.

As the extract of strain of ly₅ was capable of reducing aminoadipate in the presence of ATP, Mg^{2+} and NADPH without forming any aminoadipic semialdehyde (Fig. 2), it was thought that the cell-free extract of the mutant could be used for the isolation of the postulated aminoadipic semialdehyde adenylate (Scheme 1). The cell-free extract of

Table 4. Conversion of the product isolated from the activation of amino adipate into amino adipic semialdehyde by the cell-free extract of *Saccharomyces* strain WL-1, Mg^{2+} and NADPH

The isolated product from the activation of amino adipate (approx. 0.8 mg, which gave approx. 15000 c.p.m.) was incubated in the presence of 0.5 μ mol of NADPH, 2.0 μ mol of $MgCl_2 \cdot 6H_2O$, 100 μ mol of tris-HCl buffer, pH 8.0, and 3 mg of cell-free extract of strain WL-1 in a total volume of 1.0 ml. After incubation at 30°C for 90 min the products formed were isolated by fractionation of the reaction mixture on a Dowex 1 (formate form) column as described in the Materials and Methods section. The isolated materials were identified by paper chromatography with carrier compounds.

Cell-free extract	Omission from reaction mixture	Radioactivity (c.p.m.)		
		Amino adipic semialdehyde	Amino adipate	Unidentified product
Strain WL-1	None	10892	3920	1920
Strain WL-1	Mg^{2+}	1530	5422	353
Strain WL-1 (heated)	None	428	932	210

strain ly_s was therefore incubated in the presence of ATP, Mg^{2+} and NADPH with amino[6- ^{14}C]-adipate for 2 h at 30°C. The reaction mixture was then chromatographed on a Dowex 1 (formate form) column and the adsorbed materials were eluted with formic acid as described above. Although a new radioactive material did appear with 5.0M-formic acid when compared with the control experiment with heat-inactivated cell-free extract of strain ly_s , the amount recovered was extremely small (Table 3). Less than 0.03% of the total amino adipate was converted into this compound. Further, the amounts of NADPH oxidized (Fig. 2) and of product formed after the reaction in the presence of extract of strain ly_s could not be correlated. These results indicate that the product is very unstable and that most of the material was lost during the isolation process. Since no other radioactive compound, apart from amino adipate, was eluted from the ion-exchanger, it is possible that the reaction product was broken down to amino adipate.

Tests for the conversion of the radioactive material eluted with 5.0M-formic acid into amino adipic semialdehyde by cell-free extract of strain WL-1 in the absence of NADPH and for the presence of adenylate in it were not conclusive because of the extremely small yield of the material obtained from the reaction mixture.

DISCUSSION

These studies indicate that the reduction of amino adipate to amino adipic semialdehyde is a three-step process. The first and second steps require ATP and NADPH respectively besides Mg^{2+} , but the third step requires no cofactor. Contrary to the postulate that the third step was a non-enzymic reaction (Sagisaka & Shimura, 1962), it was found that this step is also an enzymic one.

Although the chemical structure of the compound formed by the extract of strain WL-1 in the presence of amino adipate, ATP and Mg^{2+} has not yet been determined, preliminary studies of the chemical properties indicate it to be an adenyl derivative of amino adipate. Accumulation of an adenyl derivative of amino adipate by a lysine auxotroph of *Saccharomyces* that was also unable to utilize amino adipate for lysine biosynthesis has been reported (Mattoon, Moshier & Kreiser, 1961). In contrast with their findings the ly_s mutant grown in the presence of amino adipate does not accumulate any adenyl derivative of amino adipic semialdehyde in the medium (A. K. Sinha & J. K. Bhattacharjee, unpublished work). It was found that the reaction product of the cell-free extract of strain ly_s incubated in the presence of amino adipate, ATP, Mg^{2+} and NADPH was very unstable and broke down to amino adipate in the absence of the enzyme for the cleavage reaction. Consequently it is not surprising that the mutant ly_s does not accumulate any adenyl derivative of amino adipate, provided it was assumed that the reaction product in strain ly_s was an adenyl derivative of amino adipic semialdehyde. The possibility that certain lysine-free mutants, incapable of utilizing amino adipate for lysine biosynthesis, could accumulate adenyl derivatives of the amino acid does, of course, exist in those cases where the genetic lesion is in the reduction of the amino acid adenylate (Scheme 1).

The amount of the product formed by the cell-free extract of strain WL-1 after the activation reaction was very small and the reaction ceased after a short time (Fig. 1). These findings, together with the fact that no adenyl derivative of amino adipate could be detected in the complete reaction mixture of the extract of strain WL-1, indicate that the products of the activation and reduction reactions were continuously used for amino adipic semialdehyde formation.

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