Partial Purification and Some Properties of Homoserine O-Acetyltransferase of a Methionine Auxotroph of Saccharomyces cerevisiae

SHUZO YAMAGATA

Department of Biology, Faculty of General Education, Gifu University, Gifu 501-11, Japan

Received 15 December 1986/Accepted 27 April 1987

A wild-type strain and six methionine auxotrophs of Saccharomyces cerevisiae were cultured in a synthetic medium supplemented with 0.1 mM L-cysteine or L-methionine and analyzed for the synthesis of homoserine O-acetyltransferase (EC 2.3.1.31). Among them, four mutant strains exhibited enzyme activity in cell extracts. Methionine added to the synthetic medium at concentrations higher than 0.1 mM repressed enzyme synthesis in two of these strains. The enzyme was partially purified (3,500-fold) from an extract of a mutant strain through ammonium sulfate fractionation and chromatography on columns of DEAE-cellulose, Phenyl-Sepharose C1-4B, and Sephadex G-150. The enzyme exhibited optimal pH at 7.5 for activity and at 7.8 for stability. The reaction product was ascertained to be O-acetyl-L-homoserine by confirming that it produced L-homocysteine in an O-acetyl-L-homoserine sulfhydrylase reaction. The Km for L-homoserine was 1.0 mM, and for acetyl coenzyme A it was 0.027 mM. The molecular weight of the enzyme was estimated to be approximately 104,000 by Sephadex G-150 column chromatography and 101,000 by sucrose density gradient centrifugation. The isoelectric point was at pH 4.0. Of the hydroxy amino acids examined, the enzyme showed reactivity only to L-homoserine. Succinyl coenzyme A was not an acyl donor. In the absence of L-homoserine, acetyl coenzyme A was decacylated by the enzyme, with a Km of 0.012 mM. S-Adenosylmethionine and S-adenosylhomocysteine slightly inhibited the enzyme, but methionine had no effect.

It is well established that in microorganisms other than a few enteric bacteria O-acetyl-L-homoserine is an essential member of the biosynthetic pathway from L-homoserine to L-methionine (9, 23). In many cases, O-acetylhomoserine synthesizes cystathionine with L-cysteine through the cystathionine γ-synthase reaction. Cystathionine is then cleaved through the β-cystathionase reaction to produce L-homocysteine. But in some microorganisms, O-acetylhomoserine can also be sulphydrolated with H2S through catalysis by O-acetylhomoserine sulphydrylase, giving rise to L-homocysteine directly (7, 17, 19, 30, 31).

The enzyme homoserine O-acetyltransferase (EC 2.3.1.31), which catalyzes transfer of the acetyl group from acetyl coenzyme A (acetyl-CoA) to the O atom of homoserine (see equation 1 below), also catalyzes the acetyl exchange reaction between O-acetylhomoserine and [14C]homoserine (see equation 2).

\[
\text{L-Homoserine} + \text{acetyl-CoA} \rightarrow \text{O-acetyl-L-homoserine} + \text{CoA} \tag{1}
\]

\[
\text{O-Acetyl-L-homoserine} + \text{L-[14C]homoserine} \rightarrow \text{L-homoserine} + \text{O-acetyl-[14C]homoserine} \tag{2}
\]

This enzyme has not yet been well characterized except for a few bacteria (16, 22, 26, 27). Shiio and colleagues (16, 22) have recently reported on feedback control by the end product methionine in the case of the enzyme from Brevibacterium flavum. Wyman et al. have described purification of the enzyme to near homogeneity (26) and also its regulation in whole cells (27) of Bacillus polymyxa. This protein is, however, subject to rapid and irreversible inactivation after extraction (26).

Regulatory properties of the enzyme of Saccharomyces cerevisiae have been reported (4, 6), based on observation of the acetyl exchange reaction (equation 2) in a crude extract. However, purification and further characterization of the enzyme from this organism have not yet been described, mainly because the amount of the protein in the cell is very small and the protein is also very unstable after extraction. To characterize the enzyme of S. cerevisiae, strains with high enzyme levels and a stabilization method were sought.

In this paper, both partial purification of the enzyme from a methionine auxotroph of S. cerevisiae and its enzymatic properties are dealt with.

MATERIALS AND METHODS

Organisms. Methionine-requiring mutant strains of the yeast S. cerevisiae were isolated by Naiki and Iwata (18) from a haploid strain (a mating type, galactose fermentable, pantothenate nonrequiring). The strains employed for investigating synthesis of the enzyme are referred to in this paper as the wild-type strain and six mutant strains derived from it; two require sulfate (strains 12 and 15), two require thiosulfate (strains 6 and 8), and two require cysteine (strains 13 and 17).

Chemicals and other materials. Synthesis of O-acetyl-L-amino acids (21) and purification of O-acetylserylne-O-acetylhomoserine sulfhydrylase of S. cerevisiae (29) were carried out as described elsewhere. DEAE-cellulose (DE-52; Whatman), Sephadex G-150 (Pharmacia Fine Chemicals), Phenyl-Sepharose C1-4B (Pharmacia), and carrier ampholite (pH 3.5 to 5.0; LKB Produkter) were obtained commercially. Bovine liver catalase, acetyl-CoA, and yeast alcohol dehydrogenase were products of Boehringer Mannheim. Rabbit muscle lactate dehydrogenase, bovine serum albumin, succinyl coenzyme A (succinyl-CoA), and O-succinyl-L-homoserine were products of Sigma Chemical Co. Mutant strains of S. cerevisiae were gifts from N. Naiki.

Culture of cells for analysis of enzyme synthesis. Cells of
each strain were cultured in a synthetic medium (18) supplemented with 0.1 mM L-cysteine or L-methionine. One 3-liter flask containing 1.5 liters of the medium was inoculated with 20 ml of a fully-grown seed culture of a given strain and then shaken vigorously at 30°C. The cells were harvested in early stationary phase by centrifugation at 3,000 × g for 5 min and subsequently washed with 500 ml of cold distilled water.

**Extraction.** Cell pellets (about 10 g from 3 liters of culture medium) were suspended in cold buffer A (50 mM Tris hydrochloride buffer, pH 7.8, containing 25% sucrose, 10 mM dL-homoserine, and 2.5 mM dithiothreitol). After the volume was adjusted to 40 ml, the suspensions were agitated in a Dyno Mill (W. A. Bachofen Maschinenfabrik, type KDL) with 65 ml of glass beads (0.25 to 0.50 mm in diameter) at 3,000 rpm for two 90-s periods with an interval of 60 s. The homogenate was centrifuged at 10,000 × g for 30 min. The supernatant solution obtained was concentrated with Aquacide II-A (Calbiochem) overnight (approximately 5 ml) and then dialyzed against 350 ml of buffer A (overnight). The dialyzed materials (approximately 5 ml) were analyzed for homoserine O-acetyltransferase activity.

**Assay of homoserine O-acetyltransferase activity.** The reaction mixture contained, in a total volume of 1.5 ml, 100 mM potassium phosphate buffer (pH 7.5), 0.65 mM 5,5'-dithiobis(2-nitrobenzoic acid), 0.13 mM acetyl-CoA, 25% sucrose, 10 mM L-homoserine, and an appropriate amount of enzyme. The reaction was carried out at 30°C. Change in absorbance at 412 nm was recorded with and without homoserine. Consumption of acetyl-CoA during the homoserine acetyltransferase reaction was calculated from the difference between the two changes in absorbance of reduced 5,5'-dithiobis(2-nitrobenzoic acid), using an E_{412} of 1 cm at 13.6 (8) (assay I). Decreases in the acetyl-CoA concentration in the reaction mixture were also determined in the absence of 5,5'-dithiobis(2-nitrobenzoic acid) by observing the decrease in absorbance at 232 nm. The change in concentration was calculated with an E_{232} at 1 cm of 4.5 for the thioether bond (1) (assay II). Assay I was always used unless stated otherwise. One unit of enzyme activity was defined as the amount catalyzing the consumption of 1 μmol of acetyl-CoA per min.

**Identification of the reaction product.** One milliliter of the reaction mixture for the homoserine O-acetyltransferase reaction, which contained 75 mU of the enzyme and increased concentrations of L-homoserine (20 mM) and acetyl-CoA (5 mM), was incubated at 30°C for 30 min. After incubation, a purified preparation of O-acetyl-serine-O-acetylmocysteine sulphydrylase (28 U) from *S. cerevisiae* (29) and 2 μmol of Trisulfide were added. The mixture was further incubated for 2 h at the same temperature. The reaction was stopped with 3% trichloroacetic acid, and the mixture was then deproteinized by centrifugation at 3,000 rpm for 5 min. The supernatant fraction was applied to a Dowex 50W-X8 column (1 by 3 cm, H⁺ form). After the column was washed with distilled water, amino acids were eluted with 3 N ammonia-water. The amino acids were then oxidized with performic acid and subsequently lyophilized by the method of Hirs (10). The lyophilized material was analyzed for the presence of L-homocysteine, a reaction product of O-acetylhomoserine sulphydrylase, by paper electrophoresis as described previously (28).

**Table 2.** Comparison of specific activities of homoserine O-acetyltransferase in methionine auxotrophs of *S. cerevisiae*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Homoserine O-acetyltransferase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/g of protein</td>
</tr>
<tr>
<td>Wild type</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>24.7</td>
</tr>
<tr>
<td>8</td>
<td>1.52</td>
</tr>
<tr>
<td>12</td>
<td>20.3</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>17</td>
<td>7.22</td>
</tr>
</tbody>
</table>

a Amount of enzyme contained in cells cultured in a total volume of 3 liters of synthetic medium (18) supplemented with 0.1 mM L-cysteine hydrochloride.

**RESULTS**

**Comparison of enzyme synthesis in strains.** Table 1 shows the result of a comparative study of the contents of the enzyme in seven yeast strains, each of which was cultured in a synthetic medium (18) supplemented with 0.1 mM L-cysteine. Synthesis of the enzyme appeared to be depressed in four auxotrophs (strains 6, 8, 12, and 17). Similar results were obtained when L-cysteine was replaced by L-methionine (data not shown). The two methionine auxotrophs which showed no enzyme activity (strains 13 and 15) might be more sensitive to repression of enzyme synthesis by these two amino acids.

**Repression.** The repressive effect of L-methionine on synthesis of the enzyme in strains 6 and 12 was investigated. Methionine added at a concentration of 1 mM completely repressed enzyme synthesis (Table 2). Thus, these strains
can be considered more sensitive to methionine repression than other strains of *S. cerevisiae* (4, 6). Enzyme activity was also lacking in cell extracts of these strains cultured in rich medium (28) (data not shown). Together with the derepression of enzyme synthesis in the four methionine auxotrophs (Table 1), this suggests that the enzyme functions in *S. cerevisiae* as a synthase of O-acetyl-L-homoserine, a precursor of homocysteine biosynthesis (4, 6).

**Purification of L-homoserine O-acetyltransferase.** Cells of mutant strain 6 were cultured in the synthetic medium containing 0.1 mM L-methionine as described in Materials and Methods. The total wet weight of the cells harvested from 74 liters of culture medium was 300 g.

**Step 1.** The cells were suspended in 700 ml of buffer B (buffer A supplemented with 1 mM EDTA) and subjected to Dyno Mill agitation as described previously (28). After the glass beads had been washed with 500 ml of buffer B, the homogenate, together with the wash fluid, was centrifuged at 10,000 rpm for 30 min.

**Step 2.** The supernatant solution obtained was fractionated with ammonium sulfate between 40% (240 g/liter) and 65% saturation (additional 165 g/liter). Precipitated proteins were collected by centrifugation at 10,000 rpm for 30 min, dissolved in a small volume of buffer B, and dialyzed against 2 liters of the same buffer for 20 h.

**Step 3.** The dialyzed material was centrifuged as above. The 280 ml of supernatant obtained was diluted to 2.8 liters with buffer B and then applied to a DEAE-cellulose column (4.2 by 7 cm) equilibrated with buffer B. The column was washed with 300 ml of buffer B containing 0.05 M NaCl, and the enzyme was eluted by increasing the concentration of NaCl to 0.2 M.

**Step 4.** This solution, 75 ml, was applied to a DEAE-cellulose column (2.6 by 30 cm) after dilution to 400 ml with buffer B, and proteins were eluted with 600 ml of a linear concentration gradient (0.05 to 0.30 M) of NaCl prepared in buffer B. Fractions containing homoserine O-acetyltransferase activity were combined (113 ml).

**Step 5.** The combined fractions were brought to 30% saturation with respect to ammonium sulfate (170 g/liter). This solution was subsequently applied to a Phenyl-Sepharose CI-4B column (2.6 by 34 cm) equilibrated with buffer B containing ammonium sulfate at 30% saturation. The enzyme was eluted at a rate of 1.7 ml/min with 600 ml of a linear ammonium sulfate concentration gradient (30 to 0% saturation) prepared in buffer B. Active fractions were combined, yielding 34 ml.

**Step 6.** This solution was concentrated with a collodion bag (Sartorius Membranfilter, SM 13200) to 6 ml and then subjected to gel filtration on a Sephadex G-150 column (2.6 by 92 cm) which was equilibrated with buffer B containing 0.05 M NaCl. The flow rate was 8 ml/h. Active fractions were combined, providing 21.5 ml.

**Step 7.** This final fraction was subjected to DEAE-cellulose column (1.5 by 23 cm) chromatography, in which the enzyme was eluted with 300 ml of a linear NaCl concentration gradient from 0.05 to 0.25 M. The flow rate was 12 ml/h, and the eluate was fractionated by 3.4-ml fractions. Table 3 summarizes the results of purification of the enzyme.

**Reaction product.** To determine whether the reaction product of the acetyltransferase was O-acetyl-L-homoserine, a reaction was carried out with the product as a substrate of O-acetylserine-O-acetylhomoserine sulfhydrylase from *S. cerevisiae* (29), and the final reaction product was analyzed by high-voltage paper electrophoresis. This product was identified as L-homocysteine, indicating that the product of the first reaction was O-acetyl-L-homoserine, since the O-acetyl-L-homoserine sulfhydrylase of *S. cerevisiae* used in the second reaction reacts specifically with O-acetyl-L-serine or O-acetyl-L-homoserine (29).

**Optimal pH.** The optimal pH for activity was determined by using the preparation obtained at step 4. Reactions were carried out at pHs from 6.0 to 10.0 with 0.05 M potassium phosphate or Tris hydrochloride buffer. The activity curve obtained showed a peak at pH 7.5 (phosphate buffer) with sharp slopes on both sides (data not shown). To determine the optimal pH for stability, the enzyme was incubated both at 30°C for 5 h and at 4°C for 23 h at pHs in the same range as above. The incubated solution, 0.2 ml, contained 4 μl of the enzyme, 0.05 M potassium phosphate or Tris hydrochloride buffer, 1 mM EDTA, 25% sucrose, and 0.1 mM dithiothreitol. The activity was determined with 0.1 ml of the incubated solution. In these experiments, the enzyme was shown to be most stable at pH 7.8 (Tris buffer).

**Substrate specificity.** The enzyme transferred an acetyl group from acetyl-CoA to L-homoserine but not to L-serine, D-serine, L-threonine, or L-hydroxyproline, each of which was added to the reaction mixture at a concentration of 10 mM in place of L-homoserine. It was also ascertained that D-homoserine neither reacted with the enzyme nor inhibited.

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**TABLE 2.** Repression of homoserine O-acetyltransferase synthesis by L-methionine

<table>
<thead>
<tr>
<th>L-Methionine (mM)</th>
<th>Mutant 6</th>
<th>Mutant 12</th>
<th>Total U*</th>
<th>Mutant 6</th>
<th>Mutant 12</th>
<th>Total U*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>11.4</td>
<td>0.47</td>
<td>23.4</td>
<td>1.11</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>28.2</td>
<td>2.90</td>
<td>26.5</td>
<td>1.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.20</td>
<td>20.6</td>
<td>2.62</td>
<td>2.47</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>13.9</td>
<td>1.82</td>
<td>0.22</td>
<td>0.033</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The amount of enzyme contained in cells cultured in 1.3 liters of the synthetic medium supplemented with L-methionine at the concentrations indicated.

**TABLE 3.** Purification of homoserine O-acetyltransferase from a methionine-requiring mutant strain of *S. cerevisiae*

<table>
<thead>
<tr>
<th>Step</th>
<th>Vol (ml)</th>
<th>Total protein (mg)</th>
<th>Homoserine acetyltransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>1,000</td>
<td>9,110</td>
<td>171</td>
</tr>
<tr>
<td>2.</td>
<td>280</td>
<td>5,180</td>
<td>141</td>
</tr>
<tr>
<td>3.</td>
<td>75</td>
<td>915</td>
<td>148</td>
</tr>
<tr>
<td>4.</td>
<td>113</td>
<td>290</td>
<td>178</td>
</tr>
<tr>
<td>5.</td>
<td>34</td>
<td>5.10</td>
<td>29.1</td>
</tr>
<tr>
<td>6.</td>
<td>21.5</td>
<td>0.518</td>
<td>19.2</td>
</tr>
<tr>
<td>7.</td>
<td>13.4</td>
<td>0.143</td>
<td>9.62</td>
</tr>
</tbody>
</table>

* Harvested from 74 liters of a synthetic medium supplemented with 0.1 mM L-methionine.
FIG. 1. Elution profiles of homoserine O-acetyltransferase (○) and acetyl-CoA deacylase (●) activities from a DEAE-cellulose column at the final step of purification. Enzyme activities were determined with 5- to 30-μl samples as described in Materials and Methods. See step 7 of the purification procedure (Table 3 and text) for conditions of chromatography.

its activity, since equal activity was observed with 0.77 mM L-homoserine and 1.54 mM DL-homoserine in the reaction mixture. The enzyme did not transfer a succinyl group from succinyl-CoA to any of the hydroxy amino acids examined.

The enzyme preparations obtained in the final step of the purification also catalyzed deacylation of acetyl-CoA in the absence of L-homoserine, and the elution curve of this activity from the DEAE-cellulose column was parallel to that of the homoserine acetyltransferase (Fig. 1). This suggests that the two activities are catalyzed by the same protein. The acetyl-CoA deacylase activity was approximately one-sixth the acetyltransferase activity under the assay conditions described in Materials and Methods. The deacylase activity tended to be inhibited by the substrate, acetyl-CoA, at concentrations higher than 0.05 mM (Fig. 2).

FIG. 2. Relationship between acetyl-CoA concentration and enzymatic activity. With the final preparation used as the enzyme, homoserine O-acetyltransferase activity (○) was determined in the presence of L-homoserine at a concentration of 2 mM; acetyl-CoA deacylase activity (●) was determined without the amino acid. Acetyl-CoA was used in both determinations at the indicated concentrations.

On the other hand, the acetyltransferase reaction showed a hyperbolic dependence on the concentration of acetyl-CoA.

The \( K_m \) for acetyl-CoA in the acetyltransferase reaction was tentatively determined from a Lineweaver-Burk plot to be 0.027 mM at fixed L-homoserine concentrations of 2 and 5 mM. At acetyl-CoA concentrations of 0.065 and 0.095 mM, the substrate (L-homoserine) saturation curves were also hyperbolic. The same procedure gave an identical \( K_m \) for L-homoserine. In the acetyl-CoA deacylase reaction, the \( K_m \) for acetyl-CoA was determined to be 0.012 mM in substrate concentrations ranging from 0.005 to 0.05 mM.

MW. The MW of the enzyme was estimated to be approximately 104,000 by gel filtration through a Sephadex G-150 column, while centrifugation on a sucrose concentration gradient of the protein resulted in an MW of 101,000, with lactate dehydrogenase of rabbit muscle as a standard. No change in MW was observed even when 5 mM L-homoserine was added during either procedure.

Isoelectric point. Electrophoresis of the enzyme gave reproducible results showing that the isoelectric point of the protein was at pH 4.0.

Inhibition. Table 4 summarizes the inhibitory effect of related amino acids on the enzyme activity of the final preparation. L-Tyrosine and L-serine neither acted as substrates of the enzyme, as mentioned above, nor inhibited the activity. The group of O-modified amino acids, including the reaction product O-acetyl-L-homoserine, inhibited the enzyme slightly. The extent of inhibition by O-acetyl-L-homoserine was similar to that observed for the Brevibacterium flavum enzyme (16). L-Methionine, the end product of the pathway in which the enzyme functions, showed no inhibitory effect at a concentration of 12 mM or higher.

Some amino acids mentioned in Table 4 also inhibited the acetyl-CoA deacylase activity: O-acetylhomoserine (40% inhibition at a concentration of 5 mM), O-succinylhomoserine (7% at 2.4 mM), homocysteine (40% at 2.2 mM), O-acetylseryl (30% at 20 mM), and S-adenosylhomocysteine (70% at 0.32 mM). These results support the idea that the same protein catalyzes both the homoserine acetyltransferase and the acetyl-CoA deacylase reaction.

Carbonyl reagents such as hydroxylamine hydrochloride (7 mM) and phenylhydrazine hydrochloride (3 mM) had no effect. Metal ions introduced into the reaction mixture in chloride forms such as MgCl₂, CaCl₂, and ZnCl₂ also had no effect on the activity at a concentration of 3 mM.

Table 4. Inhibition of homoserine O-acetyltransferase by related amino acids

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Conc (mM)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Threonine</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>L-Serine</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>O-Acetyl-L-serine</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>O-Phospho-L-homoserine</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>O-Succinyl-L-homoserine</td>
<td>4.4</td>
<td>8</td>
</tr>
<tr>
<td>O-Acetyl-L-homoserine</td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>1-Cysteine*</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>1-Homocysteine*</td>
<td>1.4</td>
<td>29</td>
</tr>
<tr>
<td>DL-Penicillamine*</td>
<td>2.0</td>
<td>51</td>
</tr>
<tr>
<td>1-Methionine</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>S-Adenosyl-L-methionine</td>
<td>0.5</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>32</td>
</tr>
<tr>
<td>S-Adenosyl-L-homocysteine</td>
<td>0.2</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>31</td>
</tr>
</tbody>
</table>

* Assay II was used for activity determination.
inhibition was observed in the presence of nucleotides (0.2 mM) such as ATP, 5'-AMP, cyclic 2',3'-AMP, cyclic 3',5'-AMP, GTP, and cyclic 3',5'-GMP.

DISCUSSION

De Robichon-Szulmajster and Cherest (6) described acetyl exchange activity (equation 2) in an extract of a wild-type cell. The specific activity of the exchange reaction was reported to be 1.1 to 1.5 M U/mg of protein. However, in this study no homoserine acetyltransferase activity (equation 1) was detected in the extract of the wild-type strain. The discrepancy can be explained by differences in assay methods (the protein concentration in their reaction mixture was 30 times higher than that in this study) and perhaps by differences in the conditions of cell culture. However, in two mutant strains used in this study, 6 and 12, the enzyme activity was 13 to 18 times higher than that observed in the extract of the wild-type strain studied by de Robichon-Szulmajster and Cherest (6). Consequently, these strains seem highly promising as enzyme sources for the study of the O-acetyltransferase from S. cerevisiae.

The cells of the two mutant strains synthesized the enzyme most actively when methionine was added to the culture medium at a concentration of 0.1 mM (Table 2). A similar result was obtained when methionine was replaced with L-cysteine (data not shown). Explanation for the repression by L-cysteine is at present impossible, since the enzyme was observed not to react with L-serine to synthesize O-acetylserine, a substrate of cysteine synthase. It is not clear why the specific activity of the enzyme in the extract of cells cultured with 0.1 mM methionine was higher than that observed in cells cultured with 0.05 mM. A methionine auxotroph of Brevibacterium flavum also appears to have an optimum methionine concentration of about 0.1 mM in minimal culture medium for synthesis of homoserine O-acetyltransferase in its extract (16). These facts seem inconsistent with the observation that methionine repressed synthesis of the enzyme at higher concentrations (Table 2). One possible explanation could be that the enzyme synthesis in this organism is not as sensitive to methionine as it has been shown to be for Bacillus polymyxa, in which synthesis is strongly repressed by methionine even at a concentration of 0.05 M (27). Also, protein synthesis in S. cerevisiae might require a higher concentration of methionine than 0.05 mM in the cell.

No significant difference was observed in the amount of enzyme synthesized by cells harvested at three different growth phases—mid-exponential, late exponential, and stationary phase (data not shown).

The homoserine O-acetyltransferase from S. cerevisiae was able to be purified approximately 3,500-fold after stabilization. This enzyme has never before been highly purified from any source in a stable form. It was ascertained in a preliminary experiment that the enzyme was very unstable when handled in a buffer which contained no substance having polyhydroxyls, such as sucrose or glycerol. L-Homoserine, the substrate, was also demonstrably effective in maintaining greater stability of the enzyme during incubation. This amino acid has also been reported to play a role in stability of the Brevibacterium flavum homoserine O-acetyltransferase (22). The final preparation in buffer B could be kept at −20°C without any measurable loss of activity for at least 3 months. Polyacrylamide slab gel electrophoresis of the final preparation, however, still exhibited at least two protein impurities, indicating that the content of this protein in the cell is extremely low. The amount of the enzyme in the cell was calculated to be approximately 1% of the amount of O-acetylserine-O-acetylhomoserine sulfhydrylase of the same organism, based on the assumptions that the purity of the final preparation obtained was approximately 30% and that a previously obtained O-acetylserine-O-acetylhomoserine sulfhydrylase preparation (purified 150-fold) was homogeneous (29).

Catalytic properties of the enzyme were also noted. The enzyme appeared to catalyze both homoserine O-acetyltransferase and acetyl-CoA decylase reactions (Fig. 1). The results summarized in Fig. 2 suggest that L-homoserine sets the enzyme molecule free to operate by receiving an acetyl group from the acetylated enzyme. Detailed kinetic studies, however, are required to understand the precise reaction mechanism. The MW has never been determined for the enzymes of other organisms, except for the MW 40,000 reported for Bacillus polymyxa homoserine O-acetyltransferase (26), but it has been suggested that this enzyme exists in an oligomeric form within the bacterial cell (27).

No concrete evidence was obtained that the activity of the enzyme is regulated through some feedback control system by metabolites related to the methionine pathway. The three amino acids having a sulfhydryl group—cysteine, homocysteine, and penicillamine—inhibited the enzyme to a similar extent, suggesting that the inhibition was the result of a nonspecific effect of the sulfhydryl compounds of these amino acids, as reported for the Brevibacterium flavum enzyme (22). L-Methionine had no inhibitory effect on the enzyme. Likewise, it has been reported to have no effect on acetyl exchange reactions in S. cerevisiae (6) and Neurospora crassa (12). In contrast, the enzymes of bacteria such as Bacillus polymyxa (26), Bacillus subtilis (3), and Brevibacterium flavum (22) are subject to end product inhibition by methionine and S-adenosylmethionine, except for one reported case with Corynebacterium glutamicum (11). In Escherichia coli, synthesis of O-succinylhomoserine in place of O-acetylhomoserine is under the control of end product inhibition (13). It is interesting that there is a clear difference between fungi and bacteria in the mechanisms for regulation of the methionine pathway.

The fact that the enzyme from S. cerevisiae was only slightly inhibited by both S-adenosylmethionine and S-adenosylhomocysteine does not exclude the possibility that the catalytic activity of this enzyme is regulated by these two amino acids. Much higher inhibition might have been observed if the amino acids had been added together to the reaction mixture. Also, Shio and Ozaki (22) have discussed the possibility that the enzymes reported to be insensitive to end product inhibition might have been desensitized to the inhibitor(s) after extraction, as shown for the Brevibacterium flavum enzyme (22).

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LITERATURE CITED

