Aminoacyl-tRNA synthetase complex in *Saccharomyces cerevisiae*

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The size distribution of aminoacyl-tRNA synthetase activity was investigated in cell extracts prepared from *Saccharomyces cerevisiae*. Bio-Gel A-5M chromatography of 105000 g supernatants separated isoleucyl-tRNA synthetase activity into three peaks, with apparent molecular masses (Da) of about 100000, 350000 and 1050 or greater. Similar results were obtained with synthetases specific for glutamic acid, serine and tyrosine. Sucrose-density-gradient centrifugation of yeast supernatants also provided evidence for the existence of synthetase complexes. These data provide the first evidence for the existence of a high-molecular-mass aminoacyl-tRNA synthetase complex in yeast, perhaps similar to those reported in higher eukaryotes.

INTRODUCTION

The aminoacyl-tRNA synthetases (aaRS) catalyse the attachment of amino acids to tRNA, a highly specific process crucial to accurate protein biosynthesis. In the cells of higher eukaryotes, these enzymes are isolated as high-molecular-mass complexes [1-3], although at present the role of this cellular organization is unclear. The size of these enzyme complexes was quite variable [4-6]; however, a stable core synthetase complex has been purified from several mammalian sources [5-9]. Although the existence of an aaRS complex is well established in higher eukaryotes, it was reported to be absent from lower eukaryotes, such as yeast, and from bacteria [1,10,11]. In a very recent review, Kisselev and Wolfison state: "The most specific feature of eukaryotic aaRSs is their ability to form large (HMW) complexes..., which distinguishes these enzymes from their prokaryotic and lower-eukaryotic counterparts" [12]. Although this conclusion has been dogmatically asserted, our laboratory reported the first evidence for an aaRS complex in *Escherichia coli* [13,14]. We showed that extracts from *E. coli* had high-molecular-mass forms of several aaRSs when chromatography was performed on Bio-Gel A-5M, whereas the enzymes appeared monomeric on Sephadex G-200. We also found that the size of the aaRS complex in *E. coli* was dependent on the method of cell disruption. We now report the first evidence showing the existence of aaRS complexes in extracts from *Saccharomyces cerevisiae*, suggesting that such an organization of these enzymes is wider spread than previously thought.

EXPERIMENTAL

Cell growth

Most experiments were carried out with *S. cerevisiae* S288C, a wild-type yeast supplied by Dr. James Blair, or with a commercial baker's yeast (see the Figure legends). Cultures were grown in medium containing 20 g of bactopeptone, 10 g of yeast extract and 20 g of glucose per litre, and were incubated at 30 °C with shaking. In larger-scale experiments, cells were grown in 10-litre batches in a New Brunswick Microferm fermentor. The same medium was used, with aeration (8 litres/min) and agitation (250 rev./min) conditions adjusted to give similar growth rates to those observed with shaken flasks.

Enzyme preparation and assay

Cells were harvested by centrifugation at 5000 g for 10 min, washed and disrupted using either a Dyno-Mill ball-mill or a French pressure cell. For the ball-mill, glass beads of 0.45-0.5 mm diam. were used under continuous flow (110 ml/min). For the French pressure cell, yeast was disrupted by two passes at 138 MPa (20000 lb/in²). With either mechanical disruption method, the temperature was maintained below 10 °C. In some experiments the following protease inhibitors were added to the lysis buffer: phenylmethanesulphonyl fluoride (0.5 mM), benzamidine (1 mM), tosyl-L-phenylalanyl-chloromethane (0.1 mM), leupeptin (4 μg/ml) and EDTA (5 mM). Extracts were clarified by centrifugation at 12000 g for 30 min. The supernatant solution was further centrifuged at 105000 g for 90 min, and this supernatant solution (S105) was used as a source of aaRS. Synthetase activity was measured as previously reported [13], each assay mixture (0.15 ml) containing: Tris/HCl, pH 7.3, 3 μmol; magnesium acetate, 1.5 μmol; ATP, pH 7, 0.3 μmol; yeast tRNA, 15 μg; 14C- or 3H-labelled amino acid (0.15-0.6 μCi), and varied levels of column or gradient fractions. Incubations were carried out at 30 °C for 20 min. A 0.1 ml sample was then added to a Whatman 3MM disc, and the discs were washed and radioactivity was determined as described previously [13].

Chromatography

Aagarose-gel chromatography was carried out by using Bio-Gel A-5M equilibrated and eluted with 0.05 M Tris/HCl, pH 7.4, 0.01 M MgCl2, 1 mM dithiothreitol, 0.15 M NaCl and 10% (v/v) glycerol (buffer A). Conditions of chromatography are given in the legends accompanying each Figure. The columns were calibrated using proteins of known molecular mass (Da): thyroglobulin, 669000; β-galactosidase, 514000; pyruvate kinase, 237000; and aldolase, 156000.

Gradient analysis

Sucrose density gradients (5-20%) in buffer A were made with the use of a Beckman Density Gradient Generator and fractionated by using an Isco model 185 Fractionator. The 13 ml gradients were centrifuged at 36000 rev./min in a SW41 rotor for 16 h. These gradient conditions were calibrated by using

Abbreviations used: aaRS, aminoacyl-tRNA synthetase; IleRS etc., isoleucyl-tRNA synthetase etc.
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some of the above protein calibration standards. Tubes were pierced and pumped from the bottom, 0.5 ml fractions being collected and assayed for synthetase activity as above.

Materials

Radioactive amino acids were obtained from DuPont NEN, Boston, MA, U.S.A., or Amersham, Arlington Heights, IL, U.S.A. Bio-Gel A-5 M was a product of Bio-Rad Laboratories, Richmond, CA, U.S.A. Protein calibration standards were purchased from Pharmacia LKB, Piscataway, NJ, U.S.A. Biochemicals were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A., and all other chemicals were ACS grade.

RESULTS

Agarose gel chromatography of yeast aaRS complex

Figure 1 shows Bio-Gel A-5M chromatography of a 105000 g supernatant solution (S105) from wild-type commercial yeast disrupted with a ball-mill. Aminoacylation assays, using a mixture of 15 14C-labelled amino acids (Figure 1a), revealed multiple peaks for total aaRS activity. A major peak of aaRS activity is seen between the 514000 molecular-mass marker and the void volume, suggesting the presence of a complex of synthetases similar in size to that found in mammalian cells. The lower two panels show that individual synthetases are eluted as multiple peaks: one at 106 Da or greater, a second at 350000 and a third at about 100000. Molecular masses for the purified yeast IleRS, SerRS and TyrRS were 124000, 95000–120000 and 80000–116000 Da respectively. Hence, these synthetases appear to exist as monomeric enzymes as well as in higher-molecular-mass complexes. Storage of this S105 preparation for 7 days at 4 °C, followed by chromatography on Bio-Gel A-5M, showed a decrease in the amount of the highest-molecular-mass complex, being replaced with low-molecular-mass activity (results not shown). This suggests that the high-molecular-mass complex may dissociate to smaller complexes and monomeric forms during purification.

We also wanted to investigate the size of the aaRS complex, using other methods of cell disruption. Figure 2 shows Bio-Gel A-5M chromatography of an S105 preparation from strain S288C, broken with a French pressure cell operated at 20000 lb/in². In this experiment, total synthetase and GluRS activities were observed as a major peak at a molecular mass of about 10⁶ Da, with minor peaks of activity at lower molecular masses.

Figure 1  Bio-Gel A-5M chromatography of an S105 preparation from commercial yeast after disruption with a Dyno-Mill, type KDL

Figure 2  Bio-Gel A-5M chromatography of an S105 preparation from Saccharomyces cerevisiae strain S288C, disrupted with a French pressure cell operated at 20000 lb/in²

A 40 ml portion of a 18.8 mg/ml solution was added to the 280 ml column (2.6 cm × 53 cm), and the protein was eluted with Buffer A plus 0.15 M NaCl at a flow rate of 12 ml/h. Phenylmethanesulfonhydrazide was present at 0.5 mM. Fractions (6 ml) were collected and assayed for total or individual synthetase activity as described in the Experimental section. Dextran Blue (db), β-galactosidase (β-gal; 514000 Da) and pyruvate kinase (pk; 237000 Da) were used as molecular-mass markers. (a) Total aminoacylation activity measured with a mixture of 15 14C-labelled amino acids. (b) [14C]Isoleucine (O) and [14C]Threonine (□) acceptance. (c) [14C]Serine (O) and [14C]Glutamic acid (□) acceptance. In panels (b) and (c) the data are plotted as a percentage of the total aminoacylation activity seen for the chromatogram, whereas measured radioactivity in c.p.m. is shown in (a).
masses. The fact that most TyrRS activity does not appear to be associated with the high-molecular-mass material (compare with Figure 1) may be due to the use of a different method of cell disruption. Taken together, the above experiments provide both sedimentation and gel-filtration evidence for the existence of high-molecular-mass aaRS complexes in yeast.

**DISCUSSION**

The present investigation challenges the accepted notion that aaRS from yeast aminoacyl-tRNA synthetases exists as free enzymes, rather than as high-molecular-mass complexes. It has been suggested that yeast aaRSs may be attached to membranes [1] or to negatively charged compounds [11], but are not associated with each other [1,12]. Schimmel and Soll point out that the aaRS complexes in micro-organisms may be quite labile [15], possibly dissociating as the result of cell disruption or subsequent manipulations associated with their isolation. At low protein concentrations the aaRS complex also binds to agarose [5], a finding we have verified. Hence the type of matrix used and the conditions of chromatography are both important factors in the successful demonstration of aaRS complexes. The present investigation indicates that, with yeast, the proportion of high-molecular-mass forms of aaRS differed, depending on the method of cell disruption and treatment of extracts. Total and individual aaRS activities were eluted in multiple peaks during Bio-Gel A-5M chromatography, with molecular masses from 10^4 to 10^6 Da. The size range of activities covers monomeric aaRS to that expected for a fairly large multienzymic complex. These high-molecular-mass aaRS complexes were seen in preparations from both strain S288C and commercial yeast. In addition to chromatographic evidence, we also show sedimentation data which confirm the existence of aaRS complexes in yeast.

The observation of multiple peaks of aaRS activities in yeast raises a number of questions. Do the monomeric synthetases exist in the cell, or are they produced as a result of conditions used in cell breakage? What is the significant of the synthetase activity of intermediate molecular mass? Are we simply observing the breakdown of the larger multienzyme complex, as was the case with the mammalian aaRS complex [3]? For example, we found that the size of the aaRS complex in *E. coli* is sensitive to the dilution of the cells during disruption (C. L. Harris, unpublished work). Sere and Matthews point out that dissociation of multienzyme complexes on dilution is expected [16], and this may be responsible for previous failures to observe aaRS complexes in yeast. The answers to the above questions will require purification and characterization of the yeast aaRS complex. Finally, several investigations have concluded that the formation of synthetase complexes occurred as an evolutionary development, exclusive to higher eukaryotes [1,2,10,12]. Our findings indicate that aaRS complexes exist in bacteria and lower eukaryotes, suggesting that a common physiological reason exists for the association of aaRS in groups. In this regard, Negrutskii and Deutscher have shown that aminoacyl-tRNA is channeled in mammalian cells, possibly being shuttled from aaRS to EF-Tu to the ribosome [17]. Such channeling indicates that the organization of the translational apparatus is important to the overall efficiency of protein synthesis. The finding of aaRS complexes in yeast suggests that such a cellular organization may be important in all organisms.

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