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YEAST MITOCHONDRIAL AND CYTOPLASMIC VALYL-tRNA SYNTHETASES

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SUMMARY

Yeast mitochondrial tRNA synthetase has been partially purified and chromatographic, catalytic and antigenic properties have been compared to the cytoplasmic homologous enzyme from yeast. No significant differences could be observed between the two enzymes with respect to their behaviour during ammonium sulfate precipitation or in chromatographic separation on DEAE cellulose, hydroxylapatite and Sephadex G 200. The Km of the two enzymes toward tRNAs from yeast mitochondria, yeast cytoplasm or E. coli are praticallly identical. The antigenic properties of the two enzymes are very similar ; antisera against either the mitochondria or the cytoplasmic enzyme lead to the inhibition of their catalytic properties. The mitochondrial ValRS is formed by a single polypeptide chain whose molecular weight is 125,000 daltons, a value very close to that of the yeast cytoplasmic enzyme.

INTRODUCTION

The existence of a mitochondrial DNA imparts to the organella a partial autonomy with regard to the nuclear biosynthetic system. This mitochondrial DNA contains information for mitochondrial protein biosynthesis, which utilizes specific tRNAs and aminoacyl-tRNA synthetases (1). Leucyl-, methionyl- and phenylalanine-tRNA synthetases from yeast mitochondria and cytoplasm are different molecular species, distinguishable by their chromatographic, catalytic and antigenic properties (2-4).

Differences between valyl-tRNA synthetases (ValRS : E.C.6.1.1.9.) from cytoplasm and from chloroplasts were reported for Phaseolus vulgaris (5) and Euglena gracilis (6, 7). The yeast $tRNA^{Val}$ from mitochondria and from cytoplasm were shown to be different species (8, 9). Very small differences with respect to the optimal pH between the mitochondrial and the cytoplasmic ValRS could be observed in yeast (8). In Tetrahymena pyriformis, on the other hand, the ValRS from mitochondria and cytoplasm could not be distinguished (10).

Abbreviations: ValRS : valyl-tRNA synthetase ; mito : mitochondrial ; cyto : cytoplasmic : Tris : Tri-(hydroxymethyl) aminomethane ; PMSF : phenylmethylsulfonylfluorophosphate ; DIFP : diisopropylfluorophosphate ; SDS : sodium dodecylsulfate.

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Since yeast cytoplasmic ValRS has been purified and some of its molecular parameters described (11, 12), we attempted the purification of the yeast mitochondrial ValRS in order to investigate its properties and compare the ValRS in the cytoplasmic and mitochondrial compartments of the cell.

MATERIAL AND METHODS :

Saccharomyces cerevisiae strain ρ^+IL8-8C was grown as described in (2). Preparation of mitochondria was performed according to (13) except that cytohelicase (Pharmindustrie, France) was used (14). The mitochondria were further purified on a sucrose gradient (15).

Valyl-tRNA synthetase assay : The 0.1 ml aminoacylation reaction mixture contained 1 µmole ATP (Na salt), 2.5 µmole $MgCl_2$, 10 µmole Tris-HCl (pH 8.0), 0.25 µmole 2-mercaptoethanol, 10 µg bovine serum albumin, 2.5 A260nm units tRNA (yeast tRNA, Boehringer Mannheim, GFR) and 10 nmole (25 µCi) of ^{3}H-labeled L-valine (CEA Saclay, France). Incubation time at + 37° was 15 min when testing during purification procedures, or 1-5 min when measuring enzymic specific activities. 80 µl of the reaction mixture was absorbed on a Whatman 3 MM paper disc (25 mm Ø) and then immerged in 5 per cent trichloroacetic acid for 10 min at + 4°C. This step was followed by two washings in 5 per cent trichloroacetic acid and two in pure ethanol. Dried paper discs were counted in 5 ml of liquid scintillation medium containing 4 g/l omnifluor (NEN, Frankfurt, GFR) in toluene. The enzymic activity unit corresponds to 1 nmole of Val-tRNA formed per min under the described assay conditions.

Polyacrylamide gel electrophoresis was performed on a 5 per cent (w/v) gel in presence of 75 mmol/l Tris-HCl pH 8.8 and 0.1 per cent (w/v) SDS as in (16). Marker proteins used for determination of apparent molecular weight consisted of β-galactosidase, phosphorylase-b, bovine serum albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor and α-lactalbumin (Pharmacia, Uppsala, Sweden).

TABLE I - Comparative properties of yeast mitochondrial and cytoplasmic valyl-tRNA synthetases

	Mitochondrial enzyme	Cytoplasmic enzyme
Percentage of $(NH_4)_2SO_4$ saturation leading to enzyme precipitation (yield)	40-70 (80 %)	40-70 (79 %)
Phosphate molarity leading to elution during hydroxylapatite chromatography	0.435	0.440
Apparent Km toward yeast total cyto tRNA	3.40×10^{-6} mol/l	3.20×10^{-6} mol/l
Calculated Km toward yeast cyto $tRNA^{Val}$	1.90×10^{-7} mol/l	1.80×10^{-7} mol/l
Apparent Km toward yeast total mito tRNA	3.30×10^{-5} mol/l	3.30×10^{-5} mol/l
Calculated Km toward yeast mito $tRNA^{Val}$	1.65×10^{-6} mol/l	1.65×10^{-6} mol/l
Apparent Km toward total E. coli tRNA	1.25×10^{-5} mol/l	1.11×10^{-5} mol/l
Per cent inhibition with antimito enzyme antibodies	100	100
Per cent inhibition with anticyto enzyme antibodies	82	93

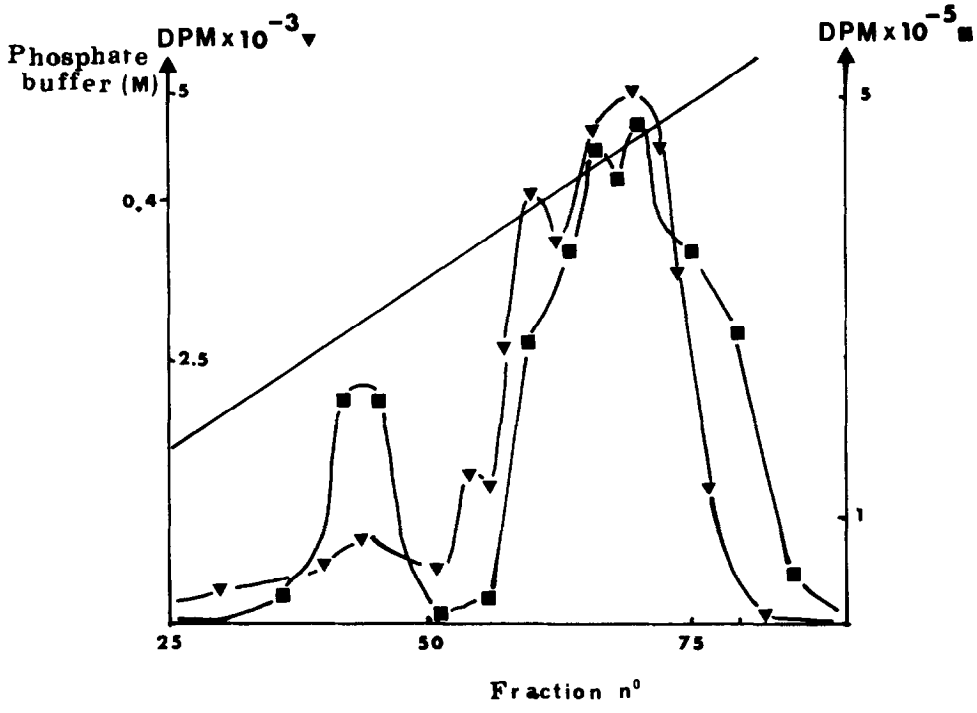

Figure 1 : Hydroxylapatite chromatogram of mitochondrial (▼) and cytoplasmic (■) val-tRNA synthetases.

Cytoplasmic valyl-tRNA synthetase from baker's yeast was prepared by the method of Kern et al. (12).

Mitochondrial valyl-tRNA synthetase was extracted from isolated yeast mitochondria. The crude extract (mitosol) was obtained by mitochondrial lysis and centrifugation as in (8, 17), PMSF or DIFP 0.1 mmol/l being added through all steps. Ammonium sulfate precipitation at 70 per cent of saturation at + 4°C was done overnight ; the precipitate was then collected by centrifugation at 50,000 x g for 45 min, dissolved and dialysed against 10 mmol/l Tris-HCl pH 7.4, 15 mmol/l $MgCl_2$ and 10 per cent (v/v) glycerol. The enzyme was separated on a DEAE-cellulose (DE-11, Whatman, Balston) column (42 x 2.4 cm) equilibrated with 10 mmol/l Tris-HCl (pH 7.4), 15 mmol/l $MgCl_2$ and 10 per cent glycerol. Elution was performed with the same buffer containing 60 mmol/l KCl ; the active enzyme elutes between 50 and 66 ml. The enzyme was precipitated by ammonium sulfate as above and further purified on a Sephadex G-200 column (55 x 16 cm). Elution was carried out with potassium phosphate 20 mmol/l (pH 7.5), 2-mercaptoethanol 5 mmol/l, $MgCl_2$ 1.5 mmol/l and 10 per cent glycerol (v/v).

Hydroxylapatite chromatography was performed as in (2). Phosphate eluting gradient was from 0 to 0.6 mol/l (pH 7.5).
Antibodies were produced in rabbits against yeast mito and cyto Val-tRNA synthetases as described previously (3, 4). Control sera were taken before immunization. 0.5 to 1.0 unit of enzyme were treated with 20 µl serum in 100 µl final volume.

Protein was determined by the method of Lowry et al. (18).

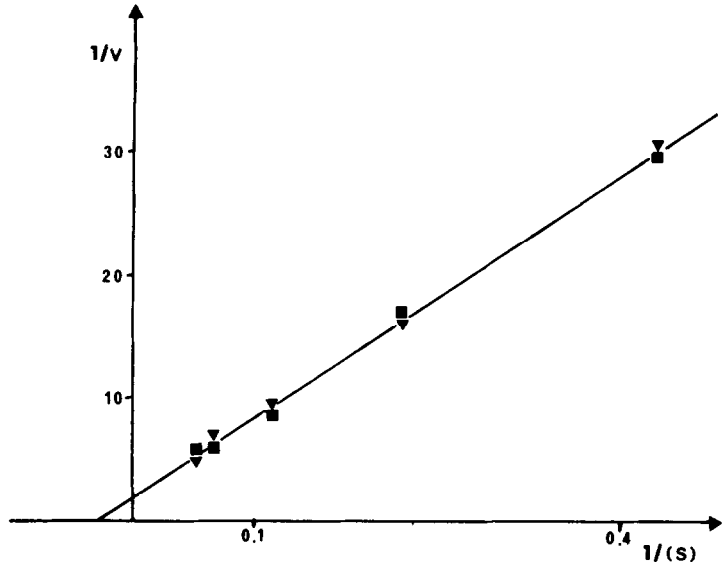

Figure 2 : Lineweaver and Burk plot of mito ValRS (▼) and cyto ValRS (■). Substrate concentration (yeast mito tRNA) ranged from 2.5-20.0 µmol/l.
(Mean molecular weight 27,000). Enzyme concentration was 0.25 unit/100 µl.

RESULTS

1) Comparative properties of the cytoplasmic and mitochondrial enzymes

Ammonium sulfate precipitation : the enzymes of the two cell compartments are equally precipitated between 40 and 70 per cent ammonium sulfate saturation at + 4°C (Table I).

Hydroxylapatite chromatography : both enzymes are retained by the column at phosphate concentrations lower than 20 mmol/l. The mitochondrial and cytoplasmic ValRS-2 are eluted with very similar phosphate concentrations of the order of 0.44 mol/l (Figure 1 and Table I). The small peak in the first eluate corresponds to both enzymes and represents degraded material (12).

Km values toward various tRNAs : apparent Km toward total yeast cyto tRNA of the two enzymes are very close, being approximately 3.3×10^{-6} mol/l. All three isoacceptors for valine in the cytoplasm are aminoacylated by the two enzymes (8), and as the $tRNA^{Val}$ in our preparation is 6 per cent, corrected Km values toward cyto $tRNA^{Val}$ are of the order of 1.9×10^{-7} mol/l for both enzymes (Table I). Affinities of the enzymes for total E. coli tRNA and for total yeast mito tRNA (Figure 2) are somewhat lower, but there is no apparent difference between the two enzymes (Table I).

Antigenic properties of the mito and cyto enzymes : the aminoacylation velocities of mito and cyto ValRS are reduced to zero when 20 µl of serum

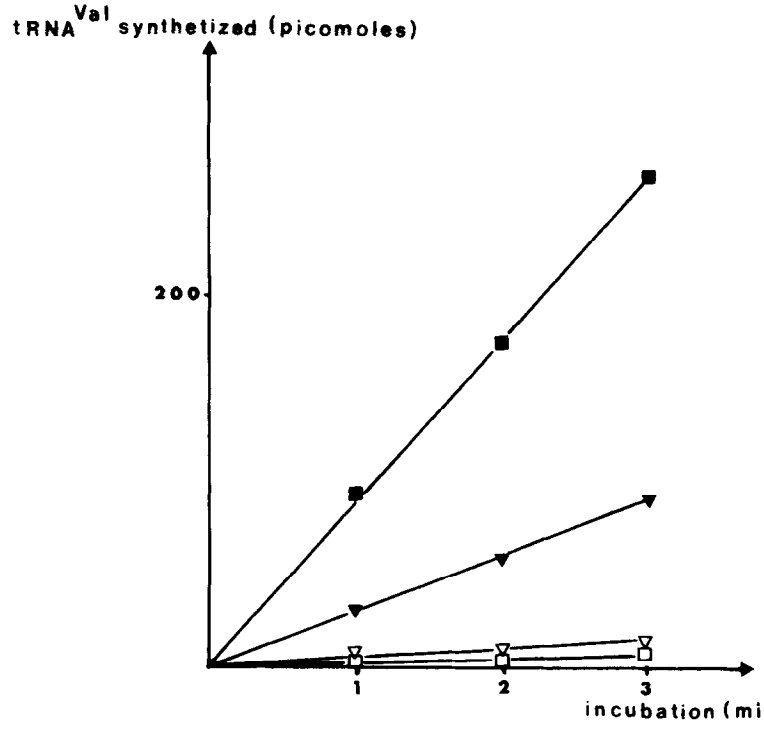

Figure 3 : Mito and cyto ValRS inhibition by antibodies in rabbits against cyto aminoacyl tRNA synthetases. 20 µl antiserum or non-immunized rabbit serum was added to the reaction mixture. Substrate was yeast cyto tRNA. Cyto ValRS was 0.85 u/100 µl, mito ValRS 0.35 u/100 µl.
Cyto ValRS + serum control (■), Cyto ValRS + antibodies (□), Mito ValRS + control serum (▼), Mito ValRS + antibodies (▽).

from rabbits immunized against mito enzymes is added instead of serum from non-immunized rabbits (control serum). The inhibition of the cyto ValRS is 93 per cent when antibodies against cytoplasmic enzymes are added. Only 82 per cent of mito ValRS is inhibited under the same conditions. (Figures 3, Table I).

2) Molecular weight determination of mitochondrial valine tRNA synthetase

The Sephadex G-200 fraction (Figure 4) containing the highest enzymic activity (500 µg protein) was divided into two parts and layered on two polyacrylamide gel cylinders. After electrophoresis one of the gels was stained by Coomassie brillant blue R and the second cut into discs of 0.6 mm thickness. Each of the latter were eluted in 100 µl aminoacylation medium and mesured for enzymic activity. In the stained gel, three protein fractions could be revealed, the major fraction being the one closest to

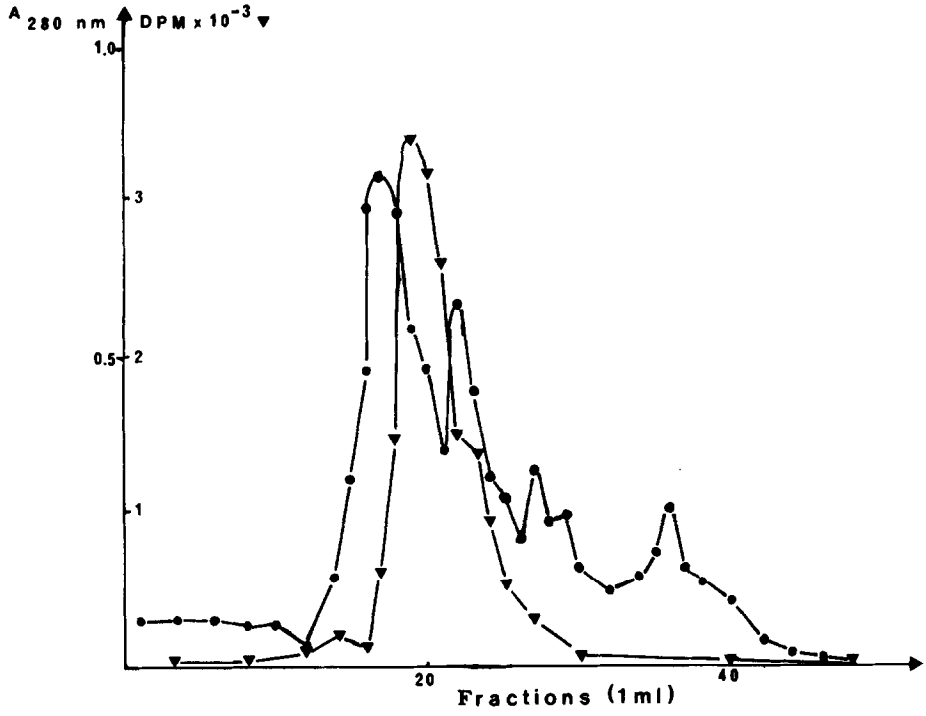

Figure 4 : Chromatogram on Sephadex G 200 (▼) mito ValRS activity ; •—• A_{280} nm.

the start. It contains almost all of the enzymic activity, although some is found in the faster running fraction as well (Figure 5). Parallel electrophoresis of standard proteins leads to a molecular weight of 125,000 for the major fraction, which contains the valine-tRNA synthetase activity.

DISCUSSION

Yeast mitochondrial Val synthetase could be partially purified in a 4-step procedure involving ammonium sulfate precipitation, DEAE cellulose chromatography, diffusion chromatography on Sephadex G 200 and electrophoresis on polyacrylamide gel. The enzymatic activity could be recovered in a fraction of the gel having an apparent molecular weight of 125,000 daltons. The enzyme consists of a single polypeptide because under dissociation conditions during electrophoresis it migrates as a single band. Yeast mitochondria contain very active proteases which are still present in low amounts throughout the various purification steps and which catalyse partial hydrolysis of the enzyme into shorter fragments with a detectable enzymatic activity and having a molecular weight around 30,000. The various properties of the yeast mitochondrial enzyme were compared to those of the yeast cyto-

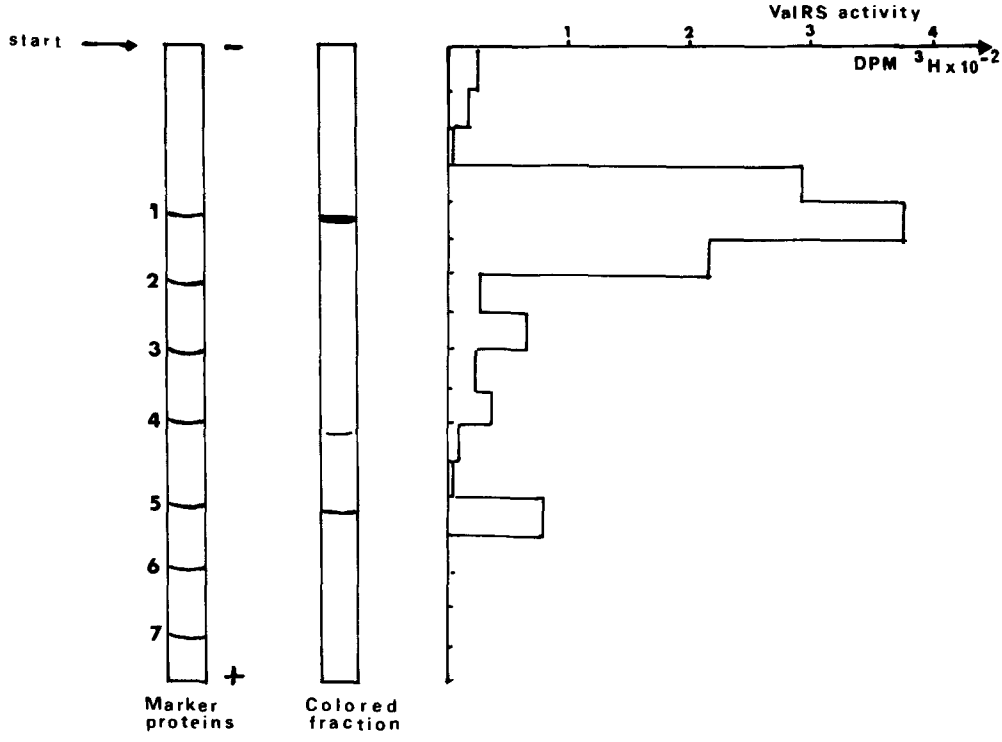

Figure 5 : Fractionation on polyacrylamide gel (5 %) of mito ValRS and marker proteins 1 : β-galactosidase, 2 : phosphorylase b, 3 : serumalbumin, 4 : ovalbumin, 5 : carbonic anhydrase, 6 : trypsin inhibitor, 7 : α-lactalbumin.

plasmic enzyme (Table I). Most of the properties studied did not show any significant difference between the two enzymes in the light of the following methods : sensitivity toward ammonium sulfate precipitation, chromatographic behaviour on DEAE cellulose, on hydroxylapatite or on Sephadex G 200 and Km values toward tRNAs from yeast mitochondria, yeast cytoplasm or E. coli. Moreover the antigenic properties are very similar. Yeast cytoplasmic ValRS has a molecular weight of 125,000 (12) and we find the same value for mitochondrial enzyme.

The two enzymes are coded by nuclear DNA since mitochondrial ValRS can be extracted from promitochondria from ρ° strain, depleted of mitochondrial DNA (19).

In other organisms such as Tetrahymena pyriformis Suyama et al. concluded that mitochondrial and cytoplasmic ValRS are identical on the basis of precipitation by tRNA at pH 5, chromatographic behaviour on hydroxylapatite, DEAE cellulose and phosphocellulose, on sedimentation analysis, heat sensi-

tivity, magnesium requirement, substrate affinity and specificity. In Tetrahymena, however, tRNA Val of the mitochondria is identical to the species in the cytoplasm and in the opinion of these authors the synthetases would be indistinguishable. In the yeast mitochondrial and cytoplasmic $tRNA^{Val}$ species are different (8) and there is no reason for the enzymes to be physically identical. It is the aim of work now in progress in this laboratory to determine the N-terminal portion of the enzymes in the two compartments of the cell.

Acknowledgements

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