PURIFICATION AND SUBUNIT STRUCTURE OF TRYPTOPHANYL tRNA SYNTHETASE (TRS) FROM BAKER'S YEAST

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1. Introduction

The proper attachment of amino acids to their cognate tRNA's by amino-acyl tRNA synthetases is one of the crucial steps for ensuring the fidelity in the translation of the genetic code. This fidelity depends on the correct recognition of both the amino acid and the corresponding tRNA by the amino-acyl tRNA synthetase. There seems to be at least one such enzyme for each of the 20 amino acids [1]. Tryptophanyl tRNA synthetase has been isolated from *E. coli* [2], beef pancreas [3], human placenta [4] and water buffalo brain [5] but this enzyme from yeast has not yet been reported. This paper deals with the purification to homogeneity and subunit structure of tryptophanyl tRNA synthetase (TRS) from baker's yeast.

2. Materials and methods

Baker's yeast was purchased locally. Unfractionated yeast tRNA was purchased from Boehringer–Mannheim. DEAE-cellulose (capacity 0.87 meq/g) was obtained from Sigma Chemicals. Hydroxyapatite was purchased from Bio-Rad Laboratories (Bio-Gel HTP, control no. 6709). L-[U-^{14}C]tryptophan (> 400 mCi/mmole) was a product of NEN. Phenyl methyl sulfonyl fluoride was obtained from Calbiochem, Inc. Protein markers were commercially obtained. All other chemicals were reagent grade.

^{t}RNA^{Tryp} was purified from crude baker's yeast tRNA by BD-cellulose chromatography following the method of Tener et al. [6] for the fractionation of brewer's yeast tRNA with the exception that the fractions from the column were directly assayed for tryptophan acceptor activity by the microassay of Cherayil and Bock [7].

2.1. *Assay procedures*

A. In column eluates the protein was determined by absorbance at 280 nm. Protein concentrations in the pooled fractions were determined by the method of Lowry et al. [8] with bovine serum albumin as standard.

B. Proteolytic enzyme activity in the extracts at each stage of purification was determined on a casein substrate by the method of Laskowsky [9].

C. Enzymic activity was assayed at the pH optimum for amino acylation in a reaction mixture which contained (in 0.5 ml) 40 μmoles of Tris-acetate (pH 7.5), 4 μmoles $MgCl_2$, 1 μmole of ATP, 2.5 μmoles of DTT, 40 μmoles of KCl, 3.6 mg of yeast tRNA or 36 μg of tRNA^{Tryp} and appropriate amounts of amino acid and enzyme. The reaction mixture was incubated at 25°C and 50 μl were withdrawn after 30 min, pipetted into filter paper disks (Schleicher and Schuell no. 593-A) which were dropped immediately into ice-cold TCA and washed and counted according to the procedure of Schmidt and Reid [10]. The optimum conditions were determined experimentally.

Gel electrophoresis was carried out essentially as described by Weber and Osborn [11] with the following minor changes. Gels were polymerized with one half the amount of cross-linker. Gels of 1.5 ml

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volume were poured into tubes of 6 mm i.d. Separation was performed at 8 mA/gel for 4 hr using bovine serum albumin, ovalbumin and β-galactosidase as markers.

2.2. *Purification of enzyme*

All operations were carried out at 4°C unless otherwise noted.

2.2.1. Steps 1 and 2: Extraction and ammonium sulfate fractionation.

The procedure of Tigerstrom and Tener [11] was followed with the following modifications. The presence of protease inhibitor, phenyl methyl sulfonyl fluoride was maintained throughout the extraction and the post mitochondrial supernatant was not spun at high speed to pellet the ribosomes but was immediately subjected to ammonium sulfate fractionation, first to 50% saturation with solid ammonium sulfate and then to 70% by addition of solid ammonium sulfate to supernatant of the 50% cut. The precipitate obtained was dissolved in minimum volume of 8 mM potassium phosphate buffer (pH 7.2), 5 mM mercaptoethanol, 1 mM EDTA and 10% propylene glycol; protease inhibitor. Inhibitor (500 μg/100 ml solution) was added and the solution dialyzed with three changes of the same buffer.

2.2.2. Steps 3 and 4: DEAE cellulose and hydroxyapatite chromatography

The dialyzed product from step 2 was applied to a DEAE-cellulose column and washed with 50 ml of the dialyzing phosphate buffer (the propylene glycol concentration at this step increased to 35%). Elution was carried out with a linear gradient from 80 to 320 mM potassium phosphate. Enzymic activity was eluted at a phosphate concentration around 0.2 M. The enzyme-containing fraction was pooled and immediately subjected to hydroxyapatite chromatography. The column was eluted with a linear gradient from 100 to 300 mM potassium phosphate. The enzyme elutes early in the gradient. The active fractions were concentrated on a mini-hydroxyapatite column and either used for analysis or stored at −70°C in 50% glycerol.

3. Results

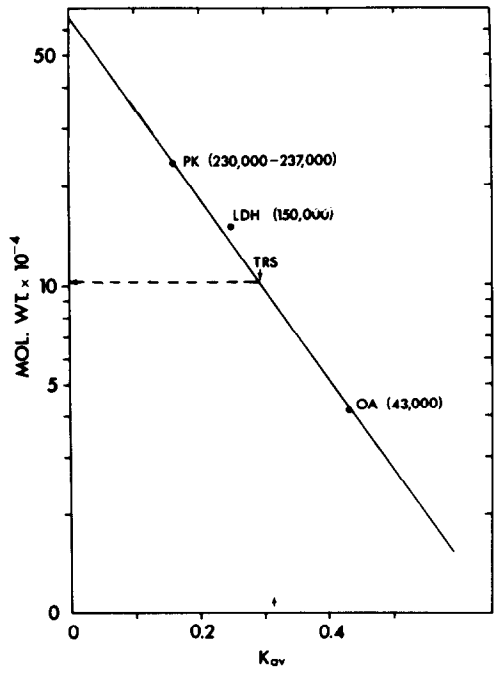
A summary of the purification procedure is given in table 1. The purification scheme for TRS includes the general features of that for phenylalanyl tRNA synthetase (PRS) [12], the main differences being the elution of the two enzymes in the DEAE-cellulose and hydroxyapatite steps. Assays indicate very low levels of proteolytic enzymic activity in the extracts.

The purified enzyme obtained after hydroxyapatite chromatography was subjected to analysis by gel filtration and electrophoresis. Fig. 1 shows the elution profile obtained by chromatography on a column of Bio gel A (1.5 m) using buffers containing 0.5 M ammonium sulfate. The preparation appears to be quite homogeneous as evidenced by a single protein peak of constant specific activity. Also shown in fig. 1 are the elution positions of known molecular weight standard proteins after chromatography on the same column. Estimation of the molecular weight of TRS from these data according to the method of Andrews [13] as shown in fig. 1 yields a value of approximately 110 000.

SDS-gel electrophoresis of the enzyme as shown in fig. 2 carried out as outlined in Materials and methods indicates the presence of a discrete subunit. Calculation of the molecular weight of the subunit by comparison to the relative mobilities as shown in fig. 2 yields a

Table 1
Purification of tryptophanyl-tRNA synthetase (TRS)

Fraction	Protein (mg)	Specific activity (cpm/mg protein)	Total activity	Purification	Recovery (%)
Postmitochondrial supernatant	1390	0.0024×10^6	3.336×10^6	1	100
$(NH_4)_2SO_4$ fraction	734	0.0032×10^6	2.348×10^6	1.33	70.4
DEAE-cellulose	33.4	0.036×10^6	1.202×10^6	15	36.2
Hydroxyapatite fraction (final enzyme)	0.525	1.5×10^6	0.788×10^6	625	23.6



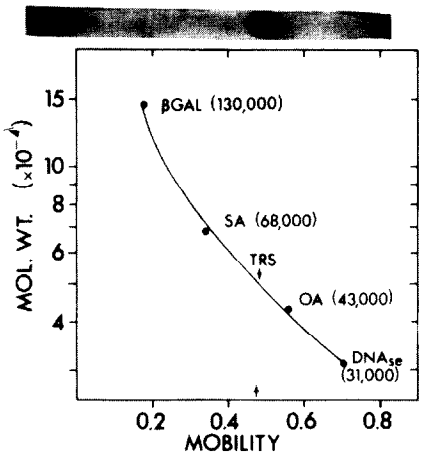


Fig. 2. SDS-gel electrophoresis and estimation of mol. wt. of the subunits of TRS. The purified enzyme was dissociated into subunits as described in the text.

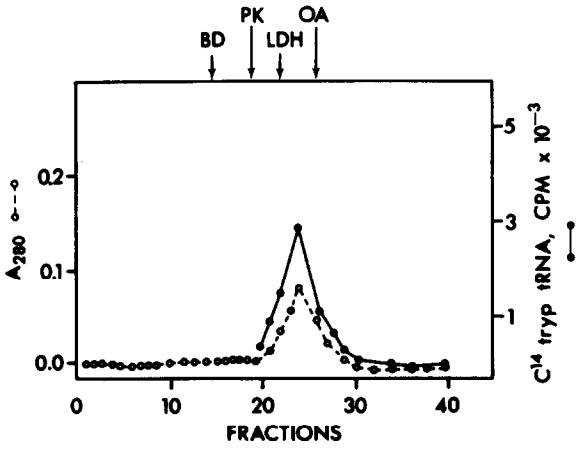


Fig. 1. Gel filtration and estimation of mol. wt. of TRS. Purified enzyme was applied to a column of Bio gel A (1 X 78) as described in ref. [12]. The arrows refer to the elution position of protein standards. K_{av} is the partition coefficient between the liquid and gel phases (see ref. [12]).

value close to 50 000. The native enzyme thus appears to be a dimer of the α_2 type as are the other tryptophanyl enzymes known [2–4], with a single possible exception [5]. The apparent molecular weight calculated from the subunit molecular weight is 100 000. Detailed studies of the stoichiometry of substrate binding and the pattern of inhibition by various agents will be reported elsewhere.

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