Purification and Some Properties of Valyl Ribonucleic Acid Synthetase from Yeast*

ULF LAGERKVIST AND JOHAN WALDENSTRÖM

From the Department of Medical Biochemistry, University of Gothenburg, Gothenburg, Sweden

SUMMARY

The purification of valyl ribonucleic acid synthetase from yeast to apparent homogeneity is described. The enzyme has a molecular weight of 112,000 and an $s_{20,w}$ of 5.5 S. It contains less than 1 nucleotide equivalent per mole of enzyme.

The mechanism by which an aminoacyl ribonucleic acid synthetase recognizes its transfer ribonucleic acid substrate constitutes one of the central problems in biochemical genetics. Obviously this problem cannot be solved without a thorough understanding of the structure of both enzyme and substrate. Although work on the structure of tRNA has yielded signal results during the last few years (1-3), very little progress has been made in the elucidation of enzyme structures. On the other hand, several aminoacyl-RNA synthetases have been obtained in highly purified form (4-15), and studies with some of them have revealed interesting information regarding the formation of enzyme-substrate complexes (16-22). In this paper we describe the purification and some properties of one of these enzymes, valyl-RNA synthetase from yeast.

EXPERIMENTAL PROCEDURE

Materials

The yeast used in this investigation (Saccharomyces cerevisiae C 836) was grown on yeast extract-glucose-salt media in the Department of Bacteriology, Karolinska Institutet, Stockholm, Sweden. The cells were harvested in the log phase, centrifuged, and stored frozen at -20°. tRNA of S. cerevisiae was prepared according to the method of von Ehrenstein and Lipmann (23). This crude tRNA preparation was used throughout this investigation. 14C-Labeled amino acids were purchased from the Radiochemical Centre, Amersham, England; Whatman DEAE-cellulose was a product of Balston, England; Amberlite IRP-64 (XE-64) was a product of Rohm and Haas, Philadelphia, Pennsylvania; and hydroxylapatite (Hypatite-C) was purchased from Clarkson Chemical Company, Williamsburg, Pennsylvania.

Methods

Enzyme assays—Valyl-RNA synthetase activity was assayed by using an incubation mixture (0.5 ml) containing 0.1 M cacodylate buffer, pH 7.0, 16 mM MgCl₂, 1 mM ATP, 0.04 mM 14C-L-valine, 10 mM tRNA nucleotide (5 to 10 μM tRNAₐᵥᵢ), 12 mM GSH, 0.2 mg per ml of bovine serum albumin, and the enzyme fraction. Incubation was carried out at 37° for 30 min. The reaction was terminated by cooling and adding 1 mg of bovine serum albumin followed by 0.05 ml of 4 M perchloric acid. After a few minutes, 2 ml of 0.4 M cold perchloric acid were added, and the precipitates were centrifuged at 1200 x g for 10 min. The pellets were washed with 3 ml of 0.4 M perchloric acid and the washing was repeated twice. The residues were dissolved in 1.0 ml of 1.4 M ammonium, and samples of 0.5 ml were plated on aluminum discs and counted as described below. One unit of valyl-RNA synthetase activity was defined as the amount of enzyme that would catalyze the formation of 1 μmole of valyl-RNA under standard conditions. Specific activity was defined as activity per mg of protein. Protein was determined according to the method of Lowry et al. (24) or calculated from the protein nitrogen obtained by a ninhydrin method (25) assuming a nitrogen content of 16% in the enzyme protein. The Lowry method was standardized by using enzyme preparations where protein nitrogen had been determined by the ninhydrin method.

Radioactivity Measurements—Determinations of 14C activity were performed in a Frieseke and Hoepfner windowless gas flow counter or in a Packard Tri-Carb liquid scintillation spectrometer, model 314 Ex-2, with Bray's scintillator solution (26).

RESULTS

Purification of Enzyme

Unless otherwise stated the operations were carried out at 0-4°. The purification procedure is summarized in Table I.

Preparation of Cell Extract and Ammonium Sulfate Precipitation—Frozen cells of S. cerevisiae C 836 (633 g of cell paste) were...
subjected to high pressure at $-25^\circ$ (27) and extracted by stirring with 2,300 ml of 0.02 M Tris buffer, pH 8.0, for 30 min. After centrifugation for 45 min at 15,000 $\times$ g, the slightly turbid extract (Fraction I, Table I) was precipitated with 0.2 volume of a 5% solution of streptomycin sulfate added dropwise with continuous stirring over a period of 15 min. Stirring was continued for another 30 min, and the resulting precipitate was removed by centrifugation for 30 min at 15,000 $\times$ g. The supernatant solution (Fraction II) was made 0.005 M with respect to GSH. To 2,300 ml of the solution were gradually added 750 g of ammonium sulfate with constant stirring, and after standing for 30 min the precipitate was centrifuged off (45 min at 15,000 $\times$ g) and discarded. To the supernatant solution (2,680 ml) were added 177 g of ammonium sulfate, and after 30 min the precipitate was collected by centrifugation at 15,000 $\times$ g for 30 min. The precipitate was dissolved in 0.02 M phosphate buffer, pH 7.5, and dialyzed overnight against 10 liters of the same buffer with one change of buffer to give a final volume of 280 ml (Fraction III).

**DEAE-cellulose Chromatography**—A column of DEAE-cellulose in phosphate form (38.5 cm x 22 cm) was prepared and equilibrated with 0.02 M phosphate buffer, pH 7.5, containing 0.05 M mercaptoethanol. The 260 ml of Fraction III were applied to the column at a rate of approximately 500 ml per hour. The enzyme was eluted by using a linear phosphate gradient. The mixing chamber contained 3,000 ml of 0.02 M phosphate buffer, pH 7.5, and the reservoir contained 3,000 ml of 0.25 M phosphate buffer, pH 6.5, both buffers contained 0.05 M mercaptoethanol. The flow rate was approximately 400 ml per hour and fractions of about 25 ml were taken. The enzyme appeared after an elution volume of about 2,700 ml. The fractions containing the major portion of the enzyme were pooled (43 ml) and precipitated with 18 g of ammonium sulfate. After 30 min, the precipitate was collected by centrifugation at 35,000 $\times$ g for 20 min.

**Hydroxyapatite Chromatography**—Enough Hypsute-C suspension to give a packed volume of approximately 20 ml was mixed with 2 g of Whatman cellulose powder and diluted to about 50 ml with 0.02 M phosphate buffer, pH 6.5, containing 0.01 M mercaptoethanol. A column of hydroxyapatite (1.1 cm x 25 cm) was prepared by pouring the suspension, divided into portions of 10 ml each, into a chromatography column with a small pad of Whatman cellulose powder at the bottom. A gentle pressure not exceeding 20 inches of water per square inch was applied in the packing of the column that was finally equilibrated with 0.02 M phosphate buffer, pH 6.5, containing 0.01 M mercaptoethanol.

The ammonium sulfate precipitate from Fraction V was dissolved in the same buffer and dialyzed against 5 liters of this buffer for 4 hours. The dialyzed fraction (10 ml) was applied to the hydroxyapatite column with the same pressure as indicated above to give a flow rate of approximately 15 ml per hour. The enzyme was eluted with a linear phosphate gradient by using 150 ml of 0.02 M phosphate buffer, pH 6.5, in the mixing chamber and 150 ml of 0.45 M phosphate buffer, pH 7.5, in the reservoir; both buffers contained 0.01 M mercaptoethanol. The flow rate was 15 ml per hour, and fractions of approximately 15 ml were taken (Fig. 1). The fractions containing the major portion of the enzyme were pooled (43 ml) and precipitated with 18 g of ammonium sulfate. After 30 min the precipitate was collected by centrifugation at 35,000 $\times$ g for 15 min. The precipitate was dissolved in a minimum amount of 0.1 M phosphate buffer, pH 7.0, containing 0.01 M GSH and dialyzed overnight against 500 ml of the same buffer. The purified enzyme (Fraction VI) could be kept frozen at $-25^\circ$ for at least 2 months without any detectable loss of activity.

**General Remarks about Purification Procedure**

Rechromatography of Fraction VI on hydroxyapatite by using the procedure described above gave a single protein peak that coincided with the enzyme activity. There was no increase in specific activity and the recovery of enzyme activity was consistently low (50% or less). Fraction VI when assayed immediately after its preparation always showed a rather low specific activity. When it was left frozen at $-25^\circ$ for 2 to 3 days, a considerable reactivation of the enzyme took place with an increase in specific activity of 4 to 5 times. This phenomenon was not dependent on the presence of protecting $-SH$ groups since it took place even in the absence of GSH. When the enzyme was tested for activity toward nine amino acids other than valine, no significant aminocyl-RNA formation was obtained (except for the presence of some lysyl-RNA synthetase activity corresponding to a contamination on a protein basis of...
Fig. 1. Chromatography of valyl-RNA synthetase on hydroxyapatite. Fraction V (80 mg of protein) was chromatographed on hydroxyapatite (1.1 cm² × 25 cm) with a linear gradient as described in the text. O represents enzyme activity, while optical density at 280 nm is shown as a continuous line. The solid bar indicates the chromatographic fractions pooled in this experiment.

0.5%, calculated from the known specific activities of the enzymes (18) (Table II).

Acrylamide gel electrophoresis of Fraction VI according to Ornstein (29) gave a single somewhat broad but apparently homogenous band. However, when large amounts of enzyme protein were analyzed (30 µg on an acrylamide gel column of 0.3 cm² × 5 cm), faint side bands were obtained indicating the presence of small amounts of impurities.

On some occasions (5 preparations out of 17), the last step in the purification of valyl-RNA synthetase produced two peaks of enzyme activity. One of them (Enzyme II) appeared in the position on the chromatogram normally occupied by valyl-RNA synthetase while the other peak was eluted earlier on the chromatogram (Enzyme I). The two peaks were always well separated from each other and showed some interesting functional differences. Enzyme I had a rather sharp pH optimum between pH 6 and 6.5 while Enzyme II showed the same broad pH optimum as normal valyl-RNA synthetase. There was no difference in the Kₐ values of the two enzymes, but the specific activity of Enzyme I was consistently lower (about 50%) than that of Enzyme II, which had the same specific activity as normal valyl-RNA synthetase. When the two enzymes were used in the assay for tRNA₉₅ (19), there was no difference in the number of valine sites recognized by them. When they were tested for ability to form the enzyme-substrate complexes, EV₉₅-AMP-Val and EV₉₅-tRNA₉₅ (19), Enzyme II behaved as the normal enzyme in this respect while Enzyme I was a consistently poor complex former. Gel filtration of the two enzyme fractions on Sephadex G-200 according to the method of Johanson and Rymo (30) indicated no difference in molecular weight. The yield of Enzyme II was consistently lower than the normal yield of valyl-RNA synthetase in preparations where only one peak of enzyme was obtained.

The successful outcome of the preparation procedure outlined above is critically dependent on the quality of the yeast employed. The yeast must be grown under rigorously controlled conditions, and only batches harvested in the log phase should be used. Attempts to prepare the enzyme from commercial yeast have been consistently unsuccessful.

| Table II
<p>| Assay of purified valyl-RNA synthetase with amino acids other than valine |</p>
<table>
<thead>
<tr>
<th>Amino acid tested</th>
<th>Valyl-RNA synthetase activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>100</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.4</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.3</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.3</td>
</tr>
<tr>
<td>Serine</td>
<td>0.3</td>
</tr>
<tr>
<td>Threonine</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Sedimentation Behavior and Determination of Molecular Weight

Fraction VI showed a single protein boundary in velocity sedimentation (Fig. 2). An s₂₀,ₐ of 5.5 S was extrapolated from sedimentation runs of two preparations of valyl-RNA synthetase at six different concentrations by using the conditions given in Fig. 2. From the sedimentation coefficient (5.4 × 10⁻¹², sec) and diffusion coefficient (4.5 × 10⁻⁷ cm² per sec) obtained on a sample (3.5 mg per ml) of one preparation, a molecular weight of 116,000 was calculated assuming a partial specific volume of 0.75 (31). Molecular weights obtained for the two preparations with the Archibald approach to equilibrium method (32) on samples of 6 mg per ml and 3.5 mg per ml, respectively, were 118,000 and 107,000.

Some General Properties of Enzyme

The enzyme was examined for bound nucleotide material. Several different enzyme preparations showed a ratio of A₂₆₀: A₉₅ ranging from 1.70 to 1.75 indicating that large fragments of RNA were not present in the preparation. A hot perchloric
FIG. 2. Velocity sedimentation of valyl-RNA synthetase. Schlieren pictures were taken at a bar angle of 50° after 16, 64, and 104 min of centrifugation at 52,640 rpm. The protein (3.5 mg per ml) had been dialyzed against 0.1 M potassium phosphate buffer, pH 7.0.

### Table III

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>$3 \times 10^{-3}$</td>
</tr>
<tr>
<td>ATP</td>
<td>$4 \times 10^{-3}$</td>
</tr>
<tr>
<td>Mg$^{++}$</td>
<td>$8 \times 10^{-3}$</td>
</tr>
<tr>
<td>tRNA$_{val}$</td>
<td>$10^{-4}$</td>
</tr>
</tbody>
</table>

acid extract of the enzymes (0.5 M perchloric acid for 10 min at 100°) showed an $A_{260}$ per mg of enzyme of 0.050 with a broad maximum around 275 nm. This would indicate that the enzyme preparation contained less than 1 nucleotide equivalent per mole of enzyme assuming a molar extinction coefficient for the hypothetical nucleotide of $10^4$ at 260 nm.

The $K_m$ values of the enzyme for its different substrates are given in Table III. The valyl-RNA synthetase had a broad pH optimum between pH 6 and 8.5 determined in a Tris-cacodylate buffer.

### DISCUSSION

This paper deals with the purification and some properties of valyl-RNA synthetase from yeast. In the preparation of this enzyme we have in about 30% of the cases obtained two separate enzyme activity peaks in the final purification step (chromatography on hydroxylapatite). One of the enzymes (Enzyme II) agrees with normal valyl-RNA synthetase both with respect to its relative position on the chromatogram and its catalytic properties (pH optimum, specific activity, recognition of valine sites in tRNA, and formation of enzyme-substrate complexes). It would therefore seem reasonable to assume that Enzyme II is identical with normal valyl-RNA synthetase. Enzyme I, on the other hand, appears earlier on the chromatogram, has a different pH optimum and a lower specific activity (50% of Enzyme II) and furthermore gives poor formation of stable enzyme-substrate complexes. The fact that Enzyme I was only found in 30% of the preparations (although it appeared, it was well separated from Enzyme II on the chromatogram), its low specific activity, and its poor ability to form stable enzyme-substrate complexes would suggest that Enzyme I is an artifact of the preparation procedure and represents a deranged form of normal valyl-RNA synthetase. This would also be consistent with the low yield of Enzyme II as compared with normal preparations where only one enzyme was obtained. A more definite conclusion regarding the status of Enzyme I will perhaps be possible when we have some information on the primary structures of Enzymes I and II.

Examination of the enzyme for nucleotide material has given no indication of the presence of oligonucleotides large enough to participate in an enzyme-substrate recognition mechanism based on complementary base pairing. The same result has been obtained by Baldwin and Berg (15) with isoleucyl-RNA synthetase from Escherichia coli.

There is no correlation between the $K_m$ values of the aminoacyl-RNA synthetases for their tRNA substrate and their ability to form stable enzyme-substrate complexes. Although the $K_m$ of valyl-RNA synthetase for tRNA$_{val}$ is approximately the same as that of lysyl-RNA synthetase from yeast for tRNA$_{lys}$, the valine enzyme forms a stable complex with tRNA$_{val}$ although the lysine enzyme does not form a stable complex with tRNA$_{lys}$. Furthermore we have recently been able to separate tRNA$_{val}$ into two fractions, tRNA$_{val}$ I and tRNA$_{val}$ II. The enzyme forms a stable complex with tRNA$_{val}$ II but not with tRNA$_{val}$ I, but the $K_m$ of the enzyme for the two tRNA$_{val}$ fractions is the same. Obviously the $K_m$ does not measure the dissociation constant of the enzyme-substrate complex, E$_{val}$-tRNA$_{val}$, at least not under our conditions of complex formation and isolation. On the other hand it should be realized that the $K_m$ values determined probably refer to enzyme-substrate complexes of the type

1 J. Waldenström, unpublished experiments.
2 U. Lagerkvist and L. Rymo, unpublished experiments.
and not to incomplete complexes like $E_{\text{Val}+\text{tRNA}_{\text{Val}}}$.

Acknowledgments—We want to thank Professor T. Laurent, Uppsala, for the ultracentrifugal analyses of the enzyme. The expert technical assistance of Mrs. Anne-Marie von Essen is gratefully acknowledged.

REFERENCES

Purification and Some Properties of Valyl Ribonucleic Acid Synthetase from Yeast
Ulf Lagerkvist and Johan Waldenström


Access the most updated version of this article at http://www.jbc.org/content/242/13/3021

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/242/13/3021.full.html#ref-list-1