Farrell, D. H., Van Nostrand, W. E., & Cunningham, D. D. (1986) *Biochem. J. 237*, 907–912.
Glenn, K. C., Carney, D. H., Fenton, J. W., II, & Cunningham, D. D. (1980) *J. Biol. Chem. 255*, 6609–6616.
Laemmli, U.K. (1970) *Nature (London) 227*, 680–685.
Low, D. A., Baker, J. B., Koonce, W. C., & Cunningham, D. D. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 2340–2344.
McGuire, E. A., & Tollefsen, D. M. (1987) *J. Biol. Chem. 262*, 169–175.
Rosenberg, R. D., & Damus, P. S. (1973) *J. Biol. Chem. 248*, 6490–6505.
Rubin, J. S., Mariz, I., Jacobs, J. W., Daughaday, W. H., & Bradshaw, R. A. (1982) *Endocrinology (Baltimore) 110*, 734–740.
Scott, R. W., & Baker, J. B. (1983) *J. Biol. Chem. 258*, 10439–10444.
Scott, R. W., Bergman, B. L., Bajpai, A., Hersh, R. T., Rodriquez, H., Jones, B. N., Barreda, C., Watts, S., & Baker, J. b. (1985) *J. Biol. Chem. 260*, 7029–7034.
Swank, R. T., & Munkres, K. D. (1971) *Anal. Biochem. 39*, 462–477.
Tollefsen, D. M., Majerus, D. W., & Blank, M. K. (1982) *J. Biol. Chem. 257*, 2162–2169.
Van Nostrand, W. E., & Cunningham, D. D. (1987) *J. Biol. Chem. 262*, 8508–8514.
Wagner, S. L., Van Nostrand, W. E., Lau, A. L., & Cunningham, D. D. (1988) *Biochemistry* (preceding paper in this issue).

Mammalian High Molecular Weight and Monomeric Forms of Valyl-tRNA Synthetase†

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ABSTRACT: Valyl-tRNA synthetase from rat liver sediments at 15.5 S with a Stokes radius of 90 Å, corresponding to a native molecular weight of 585 000. Purification of valyl-tRNA synthetase to homogeneity by a combination of conventional and affinity column chromatography yields a fully active monomeric form of valyl-tRNA synthetase with a sedimentation coefficient of 7.7 S and a Stokes radius of 45 Å. The subunit molecular weight of the monomeric valyl-tRNA synthetase is 140 000, as determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. In the presence of 400 mM KCl, the purified monomeric valyl-tRNA synthetase associates to a high molecular weight form. The high molecular weight valyl-tRNA synthetase in the homogenate can be readily converted to the monomeric form by controlled trypsinization. The kinetic parameters of the two forms are nearly identical. The results suggest that the high molecular weight valyl-tRNA synthetase is a homotypic tetramer and converts to the monomeric valyl-tRNA synthetase after the cleavage of a small peptide.

Mammalian aminoacyl-tRNA synthetases exhibit distinctly different structural organization from their prokaryotic or low eukaryotic counterparts. At least eight of the synthetases (and likely prolyl-tRNA synthetase) apparently associate as a multienzyme complex (Cirakoglu et al., 1985; Deutscher, 1984). The rest of synthetases are free soluble enzymes, similar to those in lower organisms, with the exception of valyl-tRNA synthetase (Yang et al., 1985). Mammalian valyl-tRNA synthetase has not been well studied, due to the instability of the enzyme activity and lability of the structural integrity. In the crude extract, mammalian valyl-tRNA synthetase exhibits properties similar to the multienzyme complex of aminoacyl-tRNA synthetases [e.g., see Ussery et al. (1977)]. However, valyl-tRNA synthetase does not coelute with the synthetase complex (Kellermann et al., 1982). Valyl-tRNA synthetase is the only synthetase for branched amino acids which is not associated with the synthetase complex. Several viral RNAs contain tRNA-like structure which can be charged by valyl-tRNA synthetase (Briand et al., 1976; Florentz & Giege, 1986). Valyl-tRNA synthetase has been purified from *Escherichia coli* (Yaniv & Gros, 1969), *Bacillus stearothermophilus* (Samuelsson & Lundvik, 1978), yeast (Kern et al., 1975), *Euglena gracilis* (Imbault et al., 1979), and yellow lupin seed (Jakubowski & Pawelkiewicz, 1975). Mitochondrial and cytoplasmic valyl-tRNA synthetases may be the same (Suyama & Hamada, 1978). Plant chloroplast (Colas et al., 1982) valyl-tRNA synthetase, however, appears to be different from the cytoplasmic form. Genes coding for valyl-tRNA synthetase have been isolated from *E. coli* (Skogman & Nilsson, 1984) and *Bacillus stearothermophilus* (Brand & Fersht, 1986) and yeast (Jordana et al., 1987). Recent studies of yeast valyl-tRNA synthetase in a concentrated extract suggest that a high molecular weight form of valyl-tRNA synthetase is thio-dependent, arsenite-sensitive (Black, 1983) and in oscillatory interconversion with a soluble form (Black, 1986). Valyl-tRNA synthetase has not been highly purified from any mammalian sources.

In this paper, we report the purification of valyl-tRNA synthetase from rat liver. The purified valyl-tRNA synthetase is a monomeric form of valyl-tRNA synthetase with a subunit molecular weight of 140 000 which is greater than all previously reported valyl-tRNA synthetases from various sources. The structure of the high molecular weight form of valyl-tRNA synthetase is indirectly analyzed and is consistent with being a tetramer of valyl-tRNA synthetase.

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Materials and Methods

The materials and the general methods have been described (Wahab & Yang, 1986). Buffer T contained 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 7.5 at 25 °C), 4 mM $MgCl_2$, 2 mM dithioerythritol (DTE), 10% glycerol, 0.04% NaN_3, and 0.5 mM phenylmethanesulfonyl fluoride (PMSF) which was added immediately before use from a 200 mM stock solution in isopropyl alcohol. Buffer P contained 50 mM potassium phosphate (pH 6.8), 4 mM $MgCl_2$, 2 mM DTE, 10% glycerol, 0.04% NaN_3, and 0.5 mM PMSF added prior to using it. Heparin–Sepharose was prepared by using cyanogen bromide activated Sepharose according to Cutrecasas (1970). tRNA–Sepharose was prepared as described (Remy et al., 1972).

Valyl-tRNA synthetase was assayed by measuring the rate of aminoacylation of cognate tRNA. The final reaction mixture, in 50 μL, contained 50 mM Tris-HCl (pH 7.5), 3 mM $MgCl_2$, 2 mM DTE, 2.0 mM ATP, 0.05 mM ^{14}C amino acid (50–300 μCi/μmol), 2 mg/mL bovine serum albumin (BSA), and 5 A_{260} units of tRNA. The reaction was initiated by the introduction of a limiting amount of enzyme, and immediately incubated at 37 °C for 5 min. Aliquots were spotted onto Whatman 3MM paper pads and plunged into ice-cold 5% trichloroacetic acid. The paper pads were processed for radioactivity counting as described (Johnson et al., 1979).

For sucrose gradient ultracentrifugation, samples were centrifuged at 40000 rpm in a Beckman SW 60 rotor for 6 and 14 h for 5–20% and 20–40% sucrose gradients, respectively, in a Beckman Model L5-75 ultracentrifuge. At the end of centrifugation, fractions were collected and assayed for valyl-tRNA synthetase activity.

The sedimentation coefficients were determined by the method of Martin and Ames (1961), using thyroglobulin (19 S), β-galactosidase (16 S), catalase (11.3 S), and hemoglobin (4.6 S) as internal standards.

The Stokes radius of the high molecular weight form of valyl-tRNA synthetase was determined by gel filtration on a Bio-Gel A5m (1.6 × 90 cm) column. Standards included IgM (135 Å), thyroglobulin (85 Å), β-galactosidase (69 Å), catalase (52 Å), and hemoglobin (31 Å). The standards used for the determination of the Stokes radius of the monomeric form of valyl-tRNA synthetase on Sephacryl S-200 were alcohol dehydrogenase (46 Å), hemoglobin (31 Å), and cytochrome *c* (17.4 Å). The void volumes were determined by using blue dextran 2000. The data were analyzed according to the formulation of Ackers (1967).

Purification of Valyl-tRNA Synthetase. All steps were carried out at 4 °C, unless specified otherwise. Fresh livers were minced in 2.5 volumes of buffer T with the immediate addition of 10 mM PMSF in a Waring blender for 5 s, and homogenized by using a Polytron homogenizer at setting 5 for 3 × 25 s. The broken cells were removed by centrifugation at 12000 rpm in a Sorvall SS34 rotor for 20 min.

The supernatant was decanted and brought to 30% saturation using powdered ammonium sulfate and stirred for 30 min after the addition was completed. The pellet was removed by centrifugation. The supernatant was decanted and brought to 50% ammonium sulfate and centrifuged again. The pellet was dissolved in buffer T with freshly added PMSF (10 mM) and then diluted 20-fold with buffer T. Undissolved protein and PMSF were removed by centrifugation before being loaded onto the column.

The translucent supernatant was then loaded directly onto a column (2.6 × 40 cm) of heparin–Sepharose at a flow rate of 150 mL/h. The column was washed with buffer T, and the enzyme was eluted with a linear gradient of 0–500 mM KCl (2 L) at a flow rate of 100 mL/h. Valyl-tRNA synthetase was eluted between 200 and 300 mM KCl. The active fractions were pooled and brought to 50% ammonium sulfate saturation. The protein precipitate was collected by centrifugation and redissolved in buffer P (pH 5.8).

The concentrated protein was diluted 20-fold using buffer P (pH 5.8) and loaded onto a yeast tRNA–Sepharose column (2.6 × 20 cm). The column was washed with 2 column volumes of buffer P (pH 5.8) and then with 7 column volumes of buffer P (pH 6.8) as similarly described in the purification of lupin seed valyl-tRNA synthetase (Jakubowski & Pawelkiewicz, 1975). A linear gradient of KCl (0–200 mM) in buffer P (pH 6.8) was used to elute the enzyme. Valyl-tRNA synthetase was eluted between 75 and 125 mM KCl.

The active fractions from the yeast tRNA–Sepharose column were pooled and diluted 4-fold using buffer T (pH 7.5 at 4 °C), passed through a *E. coli* tRNA–Sepharose column (0.8 × 5.3 cm), and loaded in series onto a column of (0.7 × 30 cm) heparin–Ultrogel A4R (LKB). After the loading was completed, the columns were disconnected. The heparin column was washed with buffer T and then with a linear gradient (120 mL) of KCl (0–400 mM). The enzyme eluted as a sharp peak at 0.25 M KCl.

Desalting was accomplished by centrifuging the protein sample through a gel filtration column. Bio-Gel P-6 gave a quantitative recovery, whereas 50% recovery was obtained with Sephadex G-50.

The purified enzyme is highly unstable and was stored immediately after purification in aliquots at −180 °C (stable for over 1 year). The activity of valyl-tRNA synthetase in the homogenate and in the ammonium sulfate fraction is stable and can be stored at −20 °C for 6 months or more.

Controlled Proteolysis. The sample (30–50% ammonium sulfate fraction) was diluted 5-fold with buffer T and digested with 0.01 mg/mL (800:1) *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK)-treated trypsin (Sigma) for 5 min at 4 °C or 0.02 mg/mL (400:1) for 20 min. Reactions were terminated with the addition of 0.2 mg/mL soybean trypsin inhibitor. The samples were loaded directly onto sucrose gradients and centrifuged as specified.

Determination of Kinetic Parameters. The Michaelis–Menten constants (K_m) were determined from initial velocity measurements of the aminoacylation reaction. The K_m's were determined by varying one substrate concentration, while keeping the other two substrates at saturating concentrations, and monitoring the initial velocity of aminoacylation reaction. Optimal assay conditions were used except that the concentrations of substrates were varied as specified. At least four to six different concentrations in triplicate were used. The concentration of $tRNA^{Val}$ isoacceptors was determined by aminoacylation using excess valyl-tRNA synthetase and was found to be 60 pmol/A_{260} unit. the K_m's were determined from the Eadie–Hofstee plots.

Results

Purification of Valyl-tRNA Synthetase. The purification of valyl-tRNA synthetase from rat liver is summarized in Table I. The enzyme was purified over 1000-fold with an overall yield of 5%. The specific activity of purified valyl-tRNA synthetase varies and depends on the total length of time for the purification, the presence of the protease inhibitors, and the age of the rats used. Typical yields of valyl-tRNA synthetase were 40–80 and 120–200 units/g of liver for old and young rats, respectively. In the absence of KCl, more than half of valyl-tRNA synthetase sedimented with the microsomal

Table I: Purification of Valyl-tRNA Synthetase from Rat Liver[a]

step	total protein (mg)	total act. (units)[b]	sp act. (units/mg)	yield (%)	purification (x-fold)
crude extract	7680	5250	0.68	100	1
supernatant (10 000 rpm)	6170	5000	0.81	96	1.19
30–50% $(NH_4)_2SO_4$ fractionation	3020	4050	1.34	77	1.96
heparin–Sepharose	78	2360	30.3	45	44.3
$(NH_4)_2SO_4$ concn	50	1660	36.8	32	54.1
yeast tRNA–Sepharose	2.4	500	208	9.5	306
E. coli tRNA–Sepharose	1.2	400	333	7.6	490
heparin–Ultrogel A4R	0.36	260	722	4.95	1060

[a] Four young rats gave 32 g of liver and 100 mL of crude extract, using buffer T containing 400 mM KCl and 10 mM PMSF. [b] One unit of enzyme activity is defined as the amount of enzyme which catalyzes the formation of 1 nmol of valyl-tRNA per minute.

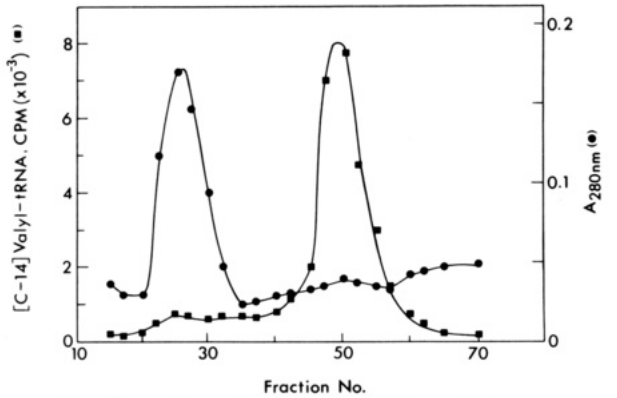

FIGURE 1: Chromatography of valyl-tRNA synthetase on yeast tRNA–Sepharose. The pooled fraction from heparin–Sepharose chromatography was precipitated with ammonium sulfate. The protein precipitate (40 mg) was diluted 20-fold using buffer P (pH 5.8) and immediately loaded onto a column (2.6 × 20 cm) of yeast tRNA–Sepharose, preequilibrated with the same buffer. The column was first washed with 400 mL of buffer P (pH 5.8) and then with 1 L of buffer P (pH 6.8). The enzyme was eluted with a linear gradient (2 L) of KCl (0–200 mM) in buffer P (pH 6.8) at a flow rate of 30 mL/h. Fractions of 20 mL were collected and assayed for valyl-tRNA synthetase activity (■) and protein concentration by the absorbance at 280 nm (●). Recovery of valyl-tRNA synthetase activity was 50%.

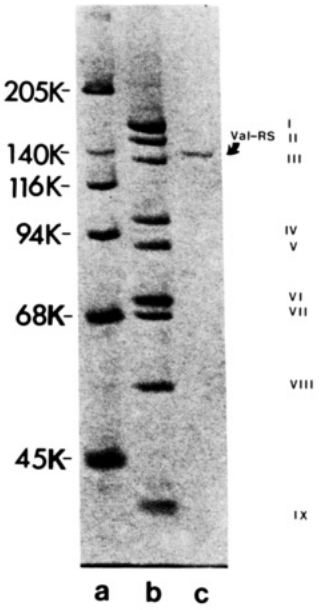

FIGURE 2: Polyacrylamide gel electrophoresis of valyl-tRNA synthetase in the presence of sodium dodecyl sulfate. Lane a shows the mixture of the molecular weight standards myosin (205 000), β-galactosidase (116 000), phosphorylase *b* (94 000), bovine serum albumin (68 000), and ovalbumin (45 000) and of valyl-tRNA synthetase which was found to have a molecular weight of 140 000. Lane b shows the purified synthetase complex for comparison. Bands II and III are isoleucyl- and leucyl-tRNA synthetases, respectively. Lane c shows the purified monomeric valyl-tRNA synthetase.

fraction during differential centrifugation. Valyl-tRNA synthetase was effectively extracted from the microsomal fraction using 400 mM KCl. Ammonium sulfate fractionation gave slightly better yield and stability than poly(ethylene glycol) fractionation.

Chromatography of the homogenate on heparin–Sepharose was very effective in the purification of valyl-tRNA synthetase. This step was rapidly carried out, to minimize endogenous proteolysis, and gave a 20-fold purification. Yeast tRNA–Sepharose was used as the second column. A pH change during chromatography was used which significantly improved the purification. A typical elution profile of the chromatography on yeast tRNA–Sepharose is shown in Figure 1. The enzyme is about 50% pure after this step as judged by sodium dodecyl sulfate (SDS) gel electrophoresis. Conventional resins for protein purification such as DEAE-cellulose, phosphocellulose, hydroxyapatite, and agarose gels invariably gave very broad peaks or very poor yields, and were avoided.

The major contaminant at this stage of purification was the high molecular weight complex of aminoacyl-tRNA synthetases, which could be absorbed on *E. coli* tRNA–Sepharose using buffer T at pH 7.5. Valyl-tRNA synthetase has effectively no affinity for *E. coli* tRNA–Sepharose under these conditions, while the synthetase complex is completely absorbed. Valyl-tRNA synthetase was then concentrated and purified to homogeneity by rechromatography on a small column of heparin–Ultrogel. The enzyme elutes as a single sharp peak between 200 and 240 mM KCl. The enzyme became progressively unstable as it is purified. The purified enzyme is extremely labile. Only by rapidly processing the steps of purification had a specific activity as high as reported here. The complete purification normally is carried out within 3–4 days. The enzyme was immediately stored at −180 °C at the end of the purification.

Figure 2 shows an SDS–polyacrylamide gel of the purified valyl-tRNA synthetase. The purified valyl-tRNA synthetase has a subunit molecular weight of 140 000, which migrates between leucyl- and isoleucyl-tRNA synthetases in the synthetase complex. The purified valyl-tRNA synthetase showed a sedimentation coefficient of 7.7 S and a native molecular weight of 145 000, as determined by sucrose gradient ultracentrifugation and gel filtration. These results indicate that the purified valyl-tRNA synthetase is a monomeric enzyme. The hydrodynamic properties of monomeric valyl-tRNA synthetase are summarized in Table II.

High Molecular Weight Valyl-tRNA Synthetase. Valyl-tRNA synthetase in the crude extract at 0 or 400 mM KCl, or in the ammonium sulfate fraction, sediments exclusively at 15.5 S, as analyzed by sucrose gradient ultracentrifugation. To ascertain that the 15.5 S form did not result from the dissociation of valyl-tRNA synthetase from the synthetase complex during ultracentrifugation, valyl-tRNA synthetase was recentrifuged on a second sucrose gradient and was found to exhibit the same sedimentation coefficient (15.5 S). These

Table II: Molecular Properties of Valyl-tRNA Synthetase from Rat Liver

	high molecular weight form	monomeric form
sedimentation coefficient (S)	15.5 ± 0.5	7.7 ± 0.3
Stokes radius (Å)	90 ± 3	45 ± 2
M_r (from Stokes radius and sedimentation coefficient)[a]	585000 ± 40000	145000 ± 11000
subunit M_r	nd[b]	140000 ± 6000
f/f_0	1.36 ± 0.05	1.09 ± 0.05
axial ratio (a/b)	6.5	2.5

[a] Assuming $\bar{v}$ is 0.73 cm³/g and the degree of hydration is 0.5 g of H_2O/g. [b] Not determined.

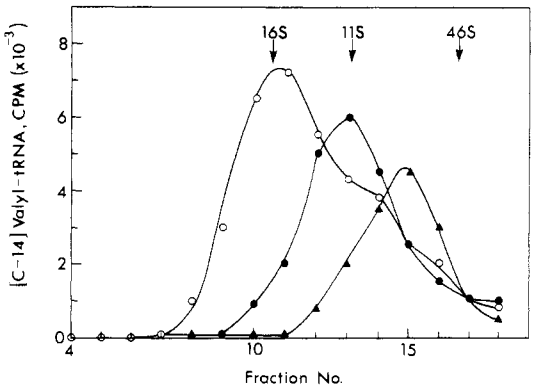

FIGURE 3: Sucrose gradient centrifugation of the different forms of valyl-tRNA synthetase. The three forms of valyl-tRNA synthetase from three parallel gradients in buffer T with 1 mg/mL bovine serum albumin and 400 mM KCl, after centrifugation on a 5–20% sucrose gradient for 8 h at 40000 rpm and 4 °C in a Beckman SW60 rotor, are shown. The high molecular weight form (○) is from the ammonium sulfate fraction, the intermediate form (●) is from the active fraction after small-scale heparin–Sepharose column chromatography, and the monomeric form (▲) is from the active fraction of preparative heparin–Sepharose column chromatography.

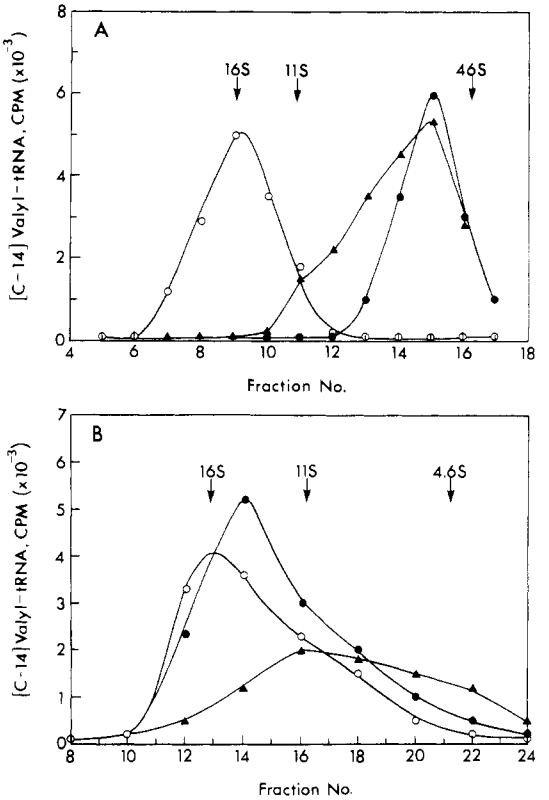

FIGURE 4: Controlled trypsinization and ribonuclease treatment of the high molecular weight valyl-tRNA synthetase. (A) Valyl-tRNA synthetase in the ammonium sulfate fraction was diluted and incubated in the presence of 0.2 mg/mL soybean trypsin inhibitor (○), in the presence of 0.01 mg/mL trypsin for 5 min at 4 °C (▲), or with 0.02 mg/mL trypsin for 20 min at 4 °C (●). The reactions were terminated by the addition of 0.2 mg/mL soybean trypsin inhibitor and immediately loaded onto three identical gradients with internal standards, including β-galactosidase (16 S), catalase (11 S), and hemoglobin (4.6 S), as indicated by the arrows. (B) Ribonuclease digestion of the high molecular weight valyl-tRNA synthetase in the presence of ribonuclease A using either free or polyacrylamide-bound forms (○). Parallel runs of the high molecular weight valyl-tRNA synthetase were in the presence of 10 mg/mL tRNA (▲) and in the absence of ribonuclease and tRNA (●). Experiments were carried out at 4 °C throughout.

results confirm the high molecular weight nature of valyl-tRNA synthetase in the crude homogenate. However, the possibility that microsomal valyl-tRNA synthetase is monomeric, and aggregates to form the 15.5 S form at 400 mM KCl, cannot be excluded.

After the preparative heparin–Sepharose column chromatography during the purification, valyl-tRNA synthetase sedimented at 7.7 S as monomer (Figure 3). When heparin–Sepharose column chromatography was carried out quickly using a small-scale preparation, most of valyl-tRNA synthetase sedimented at 11 S (Figure 3), which appears to be the dimeric form. After subsequent steps of purification, monomeric valyl-tRNA synthetase was invariably obtained.

The high molecular weight valyl-tRNA synthetase has a sedimentation coefficient of 15.5 S and a Stokes radius of 90 Å, which gave a calculated molecular weight of 585000. On the basis of the relative molecular weight, the high molecular weight valyl-tRNA synthetase is consistent with being a tetrameric form.

Fully active monomeric valyl-tRNA synthetase can be generated by controlled trypsinization (Figure 4A). Trypsinization readily gave putative dimeric (~11 S) and monomeric (7.7 S) forms, of valyl-tRNA synthetase, as analyzed by sucrose gradient ultracentrifugation. Interconversion between the 15.5 S form and the 7.7 S form of valyl-tRNA synthetase was further analyzed by gradient ultracentrifugation after a variety of treatments including ribonuclease digestion of the 15.5 S form (Figure 4B), incubation with salt (400 mM KCl) or bovine serum albumin (1 mg/mL), addition of substrates (ATP, Val, and/or liver tRNA) to the 15.5 S and 7.7 S forms, or addition of the heat-inactivated homogenate to the 7.7 S form. No change in the state of the enzyme was observed with any of the above-mentioned treatments except when the high molecular weight valyl-tRNA synthetase was incubated with tRNA or ribonuclease (Figure 4B). However, the effects appear to be complex and suggest that tRNA may also facilitate the dissociation or proteolysis of the tetramer.

Upon gel filtration on Sephacryl S-200 (molecular weight exclusion limit, 250000) in the presence of 200 mM KCl, purified monomeric valyl-tRNA synthetase gave two peaks of nearly equal amounts. One of the peaks corresponds to a molecular weight of 140000, and the other was a broad tail of activity which trailed just prior to salt. The latter peak is likely due to the interaction of mammalian synthetases with the agarose (Brevet et al., 1979). In the presence of 400 mM KCl, however, one additional peak of activity was observed near the void volume. The aggregates appear to be the high molecular weight form of valyl-tRNA synthetase. No aggregation of the monomer at 400 mM KCl was observed as analyzed by ultracentrifugation, perhaps due to the shearing forces, which were shown to partially dissociate the aminoacyl-tRNA synthetase complex (Dang & Yang, 1979).

The valyl-tRNA synthetase activity remains linear with the concentration of the enzyme in either high molecular weight

Table III: Kinetic Parameters of Valyl-tRNA Synthetase from Rat Liver[a]

	tetramer	monomer
K_m for Val (μM)	10.1 ± 1.2	8.23 ± 0.84
K_m for tRNA[b] (μM)	0.90 ± 0.08	0.92 ± 0.09
K_m for ATP (mM)	0.029 ± 0.002, 0.34 ± 0.055	0.071 ± 0.004, 0.30 ± 0.012
k_{cat} (s^{-1})	nd[c]	1.69 ± 0.13

[a] Assays were carried out under optimal conditions. See Materials and Methods for experimental details. The error ringes are indicated. [b] Corrected for valine acceptor activity of unfractionated tRNA. [c] Not determined.

or monomeric forms over 100-fold dilution, suggesting that both forms do not associate or dissociate upon dilution, or the monomeric form has the same specific activity as the tetrameric form.

Assay Conditions. Valyl-tRNA synthetase in Tris or imidazole buffer (pH 7.0) was found to be more active (2-fold) than that in phosphate buffer. The optimal concentration of Tris is 50 mM. The optimal pH is broad between pH 6 and pH 7.5. The enzyme is inhibited (by 50%) at higher pH (pH 8.5). The optimal concentration of $MgCl_2$ is 4 mM; high concentration of $MgCl_2$ (7 mM) inhibits the enzyme (by 50%). The optimal concentration of salt was 25 mM KCl; high concentration of salt inhibits the enzyme (10% residual activity at 200 mM and 50% at 100 mM). The optimal temperature is 37 °C, and no activity is detectable at 60 °C.

Kinetic Parameters. The kinetic parameters for both forms of valyl-tRNA synthetase are summarized in Table III. The catalytic constant (k_{cat}) was determined to be 1.69 s^{-1} for the monomeric valyl-tRNA synthetase. The Michaelis constants for the high molecular weight valyl-tRNA synthetase are 10 μM for Val, 0.90 μM for $tRNA^{Val}$, and 0.34 and 0.029 mM for ATP. The Michaelis constants for the monomer are 8.2 μM for Val, 0.92 μM for $tRNA^{Val}$, and 0.30 and 0.071 mM for ATP. The difference in the affinity toward ATP between the monomer and the tetramer is evident considering that the tetramer is considerably less pure than the monomer. Apparently, both forms have two ATP sites. The presence of the two ATP binding sites in the monomeric valyl-tRNA synthetase appears to be a common feature for bacterial (Yaniv & Gros, 1969), yeast (Kern et al., 1975), and mammalian valyl-tRNA synthetases.

Thermal Inactivation. The thermal lability of purified valyl-tRNA synthetase is shown in Figure 5. At 20 °C, the enzyme rapidly lost activity in buffer T. The enzyme can be best stabilized by the addition of greater than 1 mg/mL bovine serum albumin and 50% glycerol, which gave a half-life of 30 min. Other reagents were tested in an attempt to stabilize the activity, including ATP (0.2 mM), tRNA (0.25 mg/mL), Val (3 μM), all combinations of two of the substrates, DTE (0–20 mM), salt (0–400 mM KCl), ethylenediaminetetraacetic acid (EDTA) (0–1 mM), or the synthetase complex (0.2 mg/mL). However, the thermal lability persisted. Even at 4 °C, the half-life of purified valyl-tRNA synthetase is 90 min. Valyl-tRNA synthetase appears to be appreciably more labile than many other mammalian aminoacyl-tRNA synthetases.

Discussion

Valyl-tRNA synthetase apparently occurs as a high molecular weight synthetase. However, purification of the high molecular weight form of valyl-tRNA synthetase is hindered by its extreme lability and susceptibility to endogenous proteolysis. Brief trypsinization of the high molecular weight valyl-tRNA synthetase readily converts it to the monomeric form. The proteolysis of the high molecular weight form by

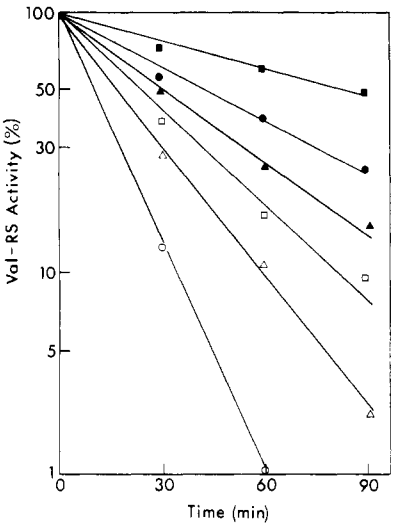

FIGURE 5: Thermal inactivation of valyl-tRNA synthetase. Purified monomeric valyl-tRNA synthetase was incubated with 0.5 mg/mL bovine serum albumin and 30% glycerol in buffer T at 4 °C (■), buffer T at 4 °C (□), buffer T with 0.5 mg/mL bovine serum albumin and 30% glycerol at 20 °C (●), buffer T at 20 °C (○), buffer T with 0.5 mg/mL bovine serum albumin at 20 °C (▲), or buffer T with 30% glycerol at 20 °C (△), and aliquots were assayed for valyl-tRNA synthetase activity.

endogenous protease appears to occur in the step of the first heparin–Sepharose column chromatography. Attempts to further control the endogenous proteolysis were unsuccessful. Interaction with tRNA or heparin may have enhanced protease susceptibility of valyl-tRNA synthetase (cf. Figure 4B). Such tRNA- or heparin-enhanced protease susceptibility has not been reported for any other synthetases.

The sedimentation coefficients and Stokes radii of both the high and low molecular weight forms were determined. The monomer showed a Stokes radius of 45 Å, a sedimentation coefficient of 7.7 S, and a subunit molecular weight of 140000. The high molecular weight valyl-tRNA synthetase has a Stokes radius of 90 Å, a sedimentation coefficient of 15.5 S, and a calculated molecular weight of 585000. These results are consistent with that the high molecular weight form is a homotypic tetramer. This is supported by the fact that the purified monomeric valyl-tRNA synthetase tends to aggregate to a high molecular weight form at high concentration of salt, which enhances the hydrophobic interactions. An intermediate 11 S form, which is consistent with being a dimeric form, was also found. We believe that the removal of a very small peptide fragment from valyl-tRNA synthetase by endogenous proteolysis resulted in the formation of the low molecular weight form. The high molecular weight valyl-tRNA synthetase is apparently not part of the multienzyme complex of aminoacyl-tRNA synthetases, which is readily separated by gel filtration or sucrose gradient centrifugation. Many different treatments were attempted; only controlled proteolysis converted the high molecular weight form to the low molecular weight form. The effects of tRNA on the high molecular weight valyl-tRNA synthetase are yet to be elucidated. Though a small fragment may have been removed from the native valyl-tRNA synthetase, the aminoacylation activity of valyl-tRNA synthetase remains unchanged. The axial ratio of the high molecular weight form is much higher than that of the monomeric from (Table II), suggesting a highly unsymmetric subunit arrangement in the tetramer.

Valyl-tRNA synthetase apparently binds to the microsomes and can be dissociated by including 400 mM KCl in the extraction buffer. A number of mammalian aminoacyl-tRNA

synthetases are known to reversibly associate with the ribosomes and microsomes [for a compilation, see Dang et al. (1982)]. The enzyme also interacts with Sephacryl-S200 and Bio-Gel A-5m. During chromatography on Bio-Gel A-5m or Sephadex G-50, recovery of valyl-tRNA synthetase was low, and a yield of greater than 50% was obtained only if 200–400 mM KCl was included in the buffer. A similar type of interaction with agarose was reported for the aminoacyl-tRNA synthetase complex (Brevet et al., 1979). It has been proposed that mammalian synthetases contain hydrophobic domains and clusters of basic amino acids (Cirakoglu & Waller, 1985), which may interact with the agarose.

Valyl-tRNA synthetase has been purified to homogeneity from a number of sources. Bacterial valyl-tRNA synthetases are monomeric (Yaniv & Gros, 1969; Samuelsson & Lundvik, 1978; Kohda et al., 1984) except for the slow growing *Mycobacterium smegmatis*, where a subunit structure of the α_4 type was reported (Natarajan & Gopinathan, 1979). Yeast valyl-tRNA synthetase was also found to be a monomer with a molecular weight of 120 000 (Kern et al., 1975). The rat liver valyl-tRNA synthetase has a higher subunit molecular weight than those previously reported for valyl-tRNA synthetase from bacteria and yeast. The subunit molecular weight of mammalian valyl-tRNA synthetase is very close to those of mammalian leucyl- and isoleucyl-tRNA synthetases (Cirakoglu et al., 1985), which are also significantly greater than their bacterial or lower eukaryotic counterparts (Bhanat et al., 1974; Baldwin & Berg, 1966; Waterson et al., 1979). The higher subunit molecular weights and higher order of structural organization appear to be common among many mammalian synthetases. The additional domains with a molecular weight of 10 000–20 000 in these synthetases are likely involved in the association of high molecular weight forms and their interaction with the protein biosynthetic machinery in mammalian cells. It appears that the additional domains in mammalian synthetases are highly susceptible to endogenous proteolysis. Similar endogenous proteolysis of methionyl-tRNA synthetase in the synthetase complex has been previously reported (Siddiqui & Yang, 1985).

The formation of the high molecular weight form of valyl-tRNA synthetase and the multienzyme complex of nine aminoacyl-tRNA synthetases in mammalian cells are thus far the most distinct structural differences between mammalian and lower eukaryotic synthetases. The present results suggest that not all high molecular weight synthetases are associated with the synthetase complex as a heterotytic multienzyme complex. Valyl-tRNA synthetase is most likely a homolytic tetrameric complex. The remaining 10 aminoacyl-tRNA synthetases appear to occur as free soluble enzymes (Yang et al., 1985), similar to those in bacteria and yeast. A high molecular weight form of valyl-tRNA synthetase, up to 5% of the total valyl-tRNA synthetase activity found in the concentrated extract of yeast (Black, 1983), may be related to the evolution of the structural organization of mammalian valyl-tRNA synthetase. Molecular cloning of mammalian valyl-tRNA synthetase should reveal the involved structural features. Comparison of valyl-tRNA synthetase with mammalian synthetases in the synthetase complex or with lower eukaryotic valyl-tRNA synthetase may provide a better understanding of the molecular nature for the differences in the subcellular organization of mammalian aminoacyl-tRNA synthetases.

Registry No. ATP, 56-65-5; Val, 72-18-4; valyl-tRNA synthetase, 9023-47-6.

References

Ackers, G. K. (1967) *J. Biol. Chem. 242*, 3237–3238.

Baldwin, A., & Berg, P. (1966) *J. Biol. Chem. 241*, 831–838.

Bhanat, O., Kucan, Z., Aoyagi, S., Lee, F. C., & Chambers, R. W. (1974) *Methods Enzymol. 29*, 547–576.

Black, S. (1983) *J. Biol. Chem. 258*, 2112–2114.

Black, S. (1986) *Science (Washington, D.C.) 234*, 1111–1114.

Brand, N. J., & Fersht, A. R. (1986) *Gene 44*, 139–142.

Brevet, A., Kellermann, O., Tonetti, H., & Waller, J. P. (1979) *Eur. J. Biochem. 99*, 551–558.

Briand, J. P., Richards, K. E., Bouley, J. P., Witz, J., & Hirth, J. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 737–741.

Cirakoglu, B., & Waller, J. P. (1985) *Eur. J. Biochem. 151*, 101–110.

Cirakoglu, B., Mirande, M., & Waller, J. P. (1985) *FEBS Lett. 183*, 185–190.

Colas, B., Imbault, P., Sarantoglou, V., & Weil, J. H. (1982) *FEBS Lett. 141*, 213–216.

Cuatrecasas, P. (1970) *J. Biol. Chem. 245*, 3059–3065.

Dang, C. V., & Yang, D. C. H. (1979) *J. Biol. Chem. 254*, 5350–5356.

Dang, C. V., Johnson, D. L., & Yang, D. C. H. (1982) *FEBS Lett. 142*, 1–6.

Deutscher, M. P. (1984) *J. Cell Biol. 99*, 373–377.

Florentz, C., & Giege, R. (1986) *J. Mol. Biol. 191*, 117–130.

Imbault, P., Sarantoglou, V., & Weil, J. H. (1979) *Biochem. Biophys. Res. Commun. 88*, 75–84.

Jakubowski, H., & Pawelkiewicz, J. (1975) *Eur. J. Biochem. 52*, 301–310.

Johnson, D. L., Dang, C. V., & Yang, D. C. H. (1980) *J. Biol. Chem. 255*, 4362–4366.

Jordana, X., Chatton, B., Paz-Weisshaar, M., Buhler, J.-M., Cramer, F., Ebel, J. P., & Fasiolo, F. (1987) *J. Biol. Chem. 262*, 7189–7194.

Kellermann, O., Tonetti, H., Brevet, A., Mirande, M., Pailliez, J. P., & Waller, J. P. (1982) *J. Biol. Chem. 257*, 11041–11048.

Kern, D., Giege, R., Robre-Saul, S., Boulanger, Y., & Ebel, J. P. (1975) *Biochimie 57*, 1167–1176.

Kohda, D., Yokoyama, S., & Miyazawa, T. (1984) *FEBS Lett. 174*, 20–23.

Martin, R. G., & Ames, B. N. (1961) *J. Biol. Chem. 236*, 1372–1379.

Natarajan, V., & Gopinathan, K. P. (1979) *Biochim. Biophys. Acta 568*, 253–263.

Remy, P., Birmele, C., & Ebel, J. P. (1972) *FEBS Lett. 27*, 134–138.

Samuelsson, T., & Lundvik, L. (1978) *J. Biol. Chem. 253*, 7033–7039.

Siddiqui, F. Q., & Yang, D. C. H. (1985) *Biochim. Biophys. Acta 828*, 177–187.

Skogman, S. G., & Nilsson, J. (1984) *Gene 30*, 219–226.

Suyama, Y., & Hamada, J. (1978) *Arch. Biochem. Biophys. 191*, 437–443.

Ussery, M. A., Tanaka, W. K., & Hardesty, B. (1977) *Eur. J. Biochem. 72*, 491–500.

Wahab, S. Z., & Yang, D. C. H. (1986) *Arch. Biochem. Biophys. 249*, 407–417.

Waterson, R. M., Gutterman, A. W., Youngblood, P., Putt, T. D., Beyersdorf, S. R., & Schusterman, M. (1979) *J. Biol. Chem. 254*, 8982–8987.

Yaniv, M., & Gros, F. (1969) *J. Mol. Biol. 44*, 1–15.

Yang, D. C. H., Garcia, J. V., Johnson, Y. D., & Wahab, S. (1985) *Curr. Top. Cell. Regul. 26*, 325–335.