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PURIFICATION AND PROPERTIES OF L-VALYL-sRNA SYNTHETASE FROM *ESCHERICHIA COLI*

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SUMMARY

L-Valyl-sRNA synthetase (L-valine:sRNA ligase (AMP), EC 6.1.1.9) has been purified from *Escherichia coli* by a procedure involving autolysis, $(NH_4)_2SO_4$ fractionation, adsorption on and elution from calcium phosphate gel, and column chromatography. The enzyme, which is about 650-fold purified over the initial cell-free extract, is homogeneous on ultracentrifugation (sedimentation coefficient 4.2 S) and on polyacrylamide-gel electrophoresis. Certain general properties of the enzyme and the effect of various metal ions on its activity have been determined. The enzyme is inhibited by *N*-ethylmaleimide and *p*-chloromercuribenzoate. Preliminary studies of the enzyme sulfhydryl groups have been carried out.

INTRODUCTION

The aminoacyl sRNA synthetases occupy a significant position in the pathway leading from free amino acids to proteins; these enzymes play an important role in determining the specificity of protein synthesis by recognizing both a particular amino acid and the corresponding sRNA acceptor. Understanding of the mechanism of this step in protein synthesis will undoubtedly require a variety of studies on the purified aminoacyl sRNA synthetases. The present work was undertaken as an effort in this direction. We have developed a method for the isolation of highly purified L-valyl-sRNA synthetase (L-valine:sRNA ligase (AMP), EC 6.1.1.9) from *Escherichia coli*. The isolated enzyme is about 650-fold purified over the original cell-free extract and is homogeneous by electrophoretic and ultracentrifugal criteria. Studies have been carried out on the specificity and other properties of the enzyme, and preliminary findings on the enzyme sulfhydryl groups have been made.

Abbreviations: sRNA, soluble RNA; PCMB, *p*-chloromercuribenzoate.

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EXPERIMENTAL

Materials

Uniformly labeled L-[^{14}C]valine was obtained from the New England Nuclear Corporation, Boston, Mass. L-Valine, L-isoleucine, DL-alloisoleucine, DL-norvaline, and DL-allothreonine were obtained from Mann Research Laboratories, New York, N.Y. DL-1-Aminobutylphosphonic acid, DL-1-amino-2 methylpropylphosphonic acid, α-aminoisobutyric acid, L-penicillamine, L-α-aminobutyric acid, and Bio-Gel P-200 were obtained from Calbiochem, Los Angeles, Calif. DL-Homoserine, DL-homocysteine, DL-isovaline, and DL-β-methylaspartic acid were obtained from Nutritional Bioch. Corp., Cleveland, Ohio. L-*tert.*-Leucine was a sample prepared by Dr. J. P. GREENSTEIN.

E. coli B was purchased from the Grain Processing Co., Muscatine, Iowa, and stored at $-20°$ until used. DEAE-cellulose and Sephadex G-25 were obtained, respectively, from Brown and Co., Keane, N. H., and Pharmacia, Uppsala, Sweden. Glass beads (diameter, 200 μ) were obtained from Minnesota Mining and Manufacturing Co., St. Paul, Minn. Alumina C-γ-gel was obtained from the Sigma Chemical Co., St. Louis, Mo. $Na_4{}^{32}P_2O_7$ was prepared from $H_3{}^{32}PO_4$ (Atomic Energy of Canada, Ltd.) as described by BERG[1]. Calcium phosphate gel was prepared by a modification* of the method of SWINGLE AND TISELIUS[2]. Salt-free hydroxylamine was prepared as described by DAVIE[3]. Polyethyleneglycol 6000 was obtained from City Chemical Corp., New York, N.Y., 2-mercaptoethanol was a product of Eastman Chemicals, Rochester, N.Y.

METHODS

Determination of enzyme activity

Enzyme activity was followed during purification by determining the rate of formation of labeled valylhydroxamate, L-[^{14}C]valine essentially, as described by LOFTFIELD AND EIGNER[4]. The reaction mixtures contained salt-free hydroxylamine (adjusted to pH 7.0 with HCl; 450 μmoles), ATP (0.75 μmole), $MgCl_2$ (0.75 μmole), L-[^{14}C]valine (0.2 μmole; 80 000 counts/min), and enzyme (added last) in a final vol. of 0.16 ml. After incubation at 37° for 30 min, a 20-μl sample was removed and applied to a strip of Amberlite cation-exchange paper (SA-2; Reeve Angel; 22 mm × 126 mm) along a line 2.5 cm from one end, and instantly exposed to a heat lamp to destroy the enzyme (keeping it at a distance of about 10 cm until dry). The lower end of the paper strips were placed in a reservoir containing 0.05 M potassium phosphate buffer (pH 7.0) in a glass tank; when the solvent moved to within 1.5 cm of the top of the strips (10 min), the strips were removed and dried. Valylhydroxamate was found in the area between 1.5 cm below the origin to 2.5 cm above the origin. All of the unreacted valine appeared in a section located 2.8 cm below the solvent front to 1.2 cm above the solvent front. The radioactive areas were counted in an automatic gas-flow counter. Controls (zero time, and without enzyme) were included in each set of determinations; less than 1% of the valine radioactivity was found in the hydroxamate area. Protein was determined by the method of LOWRY *et al.*[5] using crystalline bovine serum

* We are indebted to DR. B. HORECKER for providing the details of this procedure.

albumin as standard, and also spectrophotometrically as described by WARBURG AND CHRISTIAN[6]. A unit of enzyme activity is defined as that amount of enzyme which catalyzes the formation of 1 μmole of valylhydroxamate/h under the above condition. Specific activity is defined as the units of enzyme/mg of protein. The fractions obtained in Steps 1–3 of the purification procedure (Table I) also contain isoleucyl-sRNA synthetase, which catalyzes formation of both isoleucylhydroxamate and valylhydroxamate. To obtain the value for valylhydroxamate formation due to valyl-sRNA synthetase, the activity observed with valine was corrected by subtracting 0.55 of the value obtained with L-isoleucine (ref. 7).

Amino acid-dependent $^{32}PP_i$–ATP exchange was determined as described by BERG *et al.*[8].

TABLE I

PURIFICATION OF THE ENZYME*

Step	*Fraction*	*Protein (mg)*	*Activity*		*Yield (%)*	*Purification*
			Total (units)	*Specific (units/mg)*		
1	Cell-free extract	14 200	300	0.02	(100)	(1.0)
2	Autolysate (after centrifugation)	9 500	285	0.03	95	1.5
3	$(NH_4)_2SO_4$ (after dialysis)	1 300	208	0.16	69	8.0
4	Calcium phosphate gel eluate	400	172	0.43	57	22
5	$(NH_4)_2SO_4$ (after dialysis)	150	125	0.83	42	42
6	Calcium phosphate gel eluate	44	78	1.78	26	89
7	Calcium phosphate column	2.1	18	8.60	6	430
8	Bio-Gel P-200 column	1.15	15	13.0	5	650

* From 200 g of frozen cells; other details are given in the text.

*Purification of the enzyme**

Step 1. Preparation of cell-free extract. Partially thawed *E. coli* (200 g) was suspended in 600 ml of cold 0.10 M potassium phosphate buffer (pH 7.0) containing 0.01 M 2-mercaptoethanol. Approx. one-half of the suspension was placed in a precooled refrigerated container and the probe of a M.S.E. Ultrasonic Disintegrator, 500 W, sonicator was inserted just below the surface; the cells were sonicated at maximum power for 20 min during which time the temperature was maintained below 12°. The same procedure was followed in sonicating the remaining cell suspension. After sonication the combined sonicates were mixed with 200 ml of buffer and after thorough stirring, the mixture was centrifuged for 1 h at 15 000 $\times$ *g*.

Step 2. Autolysis of nucleic acids. The partially clarified supernatant solution was treated with EDTA to yield a final concn. of 0.001 M, and then incubated at 37° until more than 90% of the material absorbing at 260 mμ remained in the soluble phase after treatment with $HClO_4$ (final concn. 3.2%); this required about 8 h of incubation. The autolysate was allowed to stand overnight and the sediment was subsequently removed by centrifugation.

* Preliminary work on the isolation method was carried out in this laboratory in collaboration with Dr. O. J. KOEPPE.

Step 3. Ammonium sulfate fractionation. The clear supernatant solution was brought to 35% of saturation (4°) by gradual addition of solid $(NH_4)_2SO_4$; after standing for 30 min the precipitate was removed by centrifugation. The $(NH_4)_2SO_4$ concn. of the supernatant solution was then increased by adding solid $(NH_4)_2SO_4$ to 55% of satn.; after standing for 30 min, the precipitate was collected by centrifugation and the supernatant was discarded. The precipitate was dissolved in 300 ml of 0.02 M potassium phosphate buffer (pH 7.0)*. After clarification of this solution by centrifugation, $(NH_4)_2SO_4$ was added to 50% of satn. and after standing for 30 min the precipitate was collected by centrifugation and extracted 3 times with 100-ml portions of 40% satd. $(NH_4)_2SO_4$. The insoluble residue was discarded and the combined $(NH_4)_2SO_4$ extracts were brought to 55% of $(NH_4)_2SO_4$ satn. and then centrifuged. The precipitated protein was dissolved in 50 ml of 0.02 M potassium phosphate buffer (pH 7.0) and dialyzed overnight against the same buffer.

Step 4. Adsorption on and elution from calcium phosphate gel. The dialyzed solution was treated with an amount of calcium phosphate gel equivalent to one-third of the weight of protein present; after standing for 30 min at 4° the supernatant solution was recovered by centrifugation. Calcium phosphate gel equivalent to twice the original weight of protein was then added; after mixing and standing for 30 min the gel was recovered by centrifugation. The gel contained about 80% of the protein that remained after the first gel treatment. The enzyme was eluted by treating the gel successively with 50-ml portions of the following solutions: water, 0.02 M potassium phosphate buffer (pH 7.0), and 0.08 M potassium phosphate buffer (pH 7.0). Washing with each solution was repeated until the supernatant solution contained virtually no material absorbing at 280 mμ. Most of the enzyme activity was eluted with the 0.08 M phosphate buffer.

Step 5. Ammonium sulfate precipitation. The combined 0.08 M potassium phosphate buffer fractions were treated with solid $(NH_4)_2SO_4$ to obtain 47% of satn.; after standing overnight the precipitated protein was removed by centrifugation. After increasing the $(NH_4)_2SO_4$ concn. of the supernatant to 65% of satn., the precipitate was collected by centrifugation, dissolved in 10 ml of 0.02 M potassium phosphate buffer (pH 7.0), and then dialyzed overnight against the same buffer.

Step 6. Adsorption on and elution from calcium phosphate gel. The dialyzed solution was treated with an amount of calcium phosphate gel equivalent to one-third of the weight of protein, and the supernatant solution was recovered by centrifugation. Calcium phosphate gel equivalent to twice the original weight of protein was then added; after standing for 30 min the supernatant solution was recovered by centrifugation. The supernatant solution contained about 35% of the protein that remained after the first gel treatment.

Steps 7 and 8. Calcium phosphate and Bio-Gel P-200 columns. The solution was dialyzed against 0.02 M potassium phosphate buffer (pH 7.0) and then added to the top of a calcium phosphate gel-cellulose column (20 cm × 1.3 cm) previously equilibrated with the same buffer. The protein was eluted stepwise with the following solutions: 0.04 M, 0.05 M, 0.06 M, 0.07 M, and 0.08 M potassium phosphate buffers (pH 7.0). Fractions of approx. 8 ml were collected per h. The enzyme activity was eluted with 0.07 M buffer (Fig. 1) and the fractions containing the enzyme were pooled,

* 0.01 M 2-mercaptoethanol was added to all buffers used in the purification procedure.

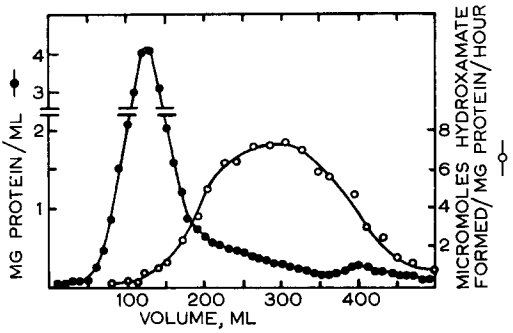


Fig. 1. Chromatography of valyl-RNA synthetase on calcium phosphate gel-cellulose. Purified valyl-RNA synthetase (455 mg, 927 units) was chromatographed on calcium phosphate gel-cellulose (1.2 cm × 21 cm), as described in the text (from large-scale preparation).

concentrated by dialysis against polyethyleneglycol 6000, and then added to the top of a Bio-Gel P-200 column (70 cm × 0.6 cm) previously equilibrated with 0.02 M potassium phosphate buffer (pH 7.0). This column was eluted with the same buffer; approx. 70% of the protein containing 75–95% of the activity was found in the first column-volume. The tubes containing most of the activity exhibited constant specific activity; these were pooled, concentrated, and frozen. Additional enzyme of similar specific activity was obtained from the other tubes after a second passage through a Bio-Gel P-200 column. The purification of the enzyme is summarized in Table I. The ratio of the absorbance of the enzyme at 280 mμ to that at 260 mμ was 1.66.

When fractions containing 100-fold purified enzyme were dialyzed against 60% sucrose containing 0.001 M glutathione and then frozen, there was no detectable loss of activity after storage for 6 months at −10°. The most highly purified enzyme has also been stored in this manner; assay after 4 months of storage indicated no loss of activity.

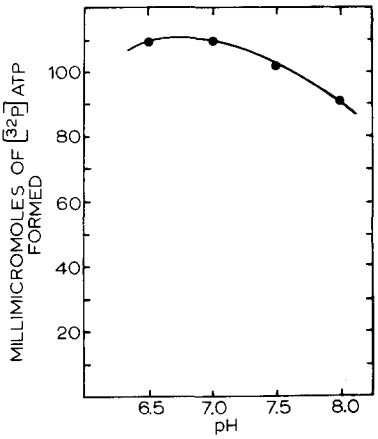
The purified enzyme sedimented in the analytical ultracentrifuge as an apparently homogeneous component; sedimentation coefficients of 4.0 and 4.3 S were obtained with protein concn. of 0.35% and 0.55%, respectively*. The enzyme migrated as a single band on electrophoresis on polyacrylamide gel at pH 8.6 (ref. 9).

Large-scale preparation of the enzyme In order to obtain larger amounts of the enzyme than can be prepared by the laboratory scale method described above, the enzyme was isolated from 16 kg batches of *E. coli*. The method used, which is described elsewhere[10], is similar to that given above; modifications were employed to permit simultaneous isolation of isoleucine sRNA synthetase[11,**].

General catalytic properties of the enzyme. The time course of hydroxamate formation was linear for at least 30 min under the conditions of assay described above. Maximal activity was observed in the pH range 6.5–7.0 in potassium phosphate buffer (Fig. 2). The K_m values for L-valine and ATP were, respectively, $2 \cdot 10^{-4}$ M and $2 \cdot 10^{-3}$ M. In the absence of added Mg^{2+}, the reaction proceeded at only 9% of that observed with an optimal Mg^{2+} concentration. The effect of various concentrations of added Mg^{2+}, Mn^{2+}, Co^{2+}, and Zn^{2+} on the reaction is given in Fig. 3. Under the con-

* We are indebted to Dr. R. H. HASCHEMEYER for these determinations.

** This work was carried out in collaboration with Dr. A. NORRIS BALDWIN.



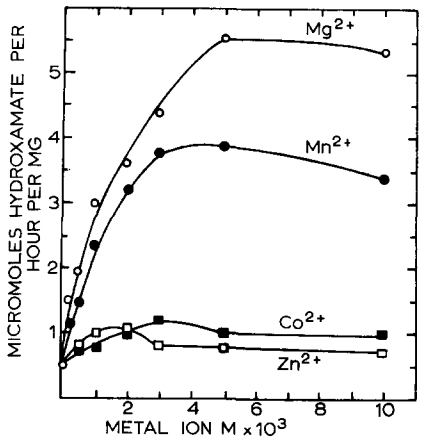


Fig. 2. Effect of pH on $^{32}PP_i$–ATP exchange. The reaction mixtures contained ATP (2 μmoles), $MgCl_2$ (5 μmoles), L-valine (2 μmoles), [^{32}P]pyrophosphate (2.1 μmoles: $1.21 \cdot 10^5$ counts/min per μmole), potassium phosphate (50 μmoles, pH 7.0), and enzyme (3.9 μg) in a final vol. of 0.5 ml. [^{32}P]ATP formation was determined as described under EXPERIMENTAL (paragraph METHODS) after incubation for 15 min at 37°.

Fig. 3. Effect of metal ions on valylhydroxamate formation. The reaction mixtures contained salt-free NH_2OH (pH 7.0, 450 μmoles), ATP (0.75 μmole), [^{14}C]valine (0.1 μmole, specific activity 1.25 μC per μmole), potassium phosphate (pH 7.0, 0.8 μmole), 2-mercaptoethanol (0.4 μmole), $MgCl_2$, $MnCl_2$, $ZnCl_2$, or $CoCl_2$ as indicated, and enzyme (5 μg) in a final vol. of 0.16 ml; incubated for 30 min at 37°.

ditions employed Mg^{2+} and Mn^{2+} were appreciably active, while much lower activity was observed with Co^{2+} and Zn^{2+}. No increase in activity was found with Ba^{2+}, Ni^{2+}, Ca^{2+}, NH_4^+, and K^+ (tested as the chlorides).

TABLE II

ACTIVITY OF THE ENZYME TOWARD VARIOUS AMINO ACIDS

The reaction mixtures consisted of enzyme (4 μg), amino acid, potassium phosphate (pH 7.0; 50 μmoles), $MgCl_2$ (5 μmoles), ATP (2 μmoles), and $Na_4{}^{32}P_2O_7$ (2.1 μmoles; 10^5 counts/min per μmole) in a final vol. of 0.5 ml. After incubation at 37° for 15 min, 0.5 ml of 7% $HClO_4$ was added and the formation of $AT^{32}P$ was determined as described under EXPERIMENTAL (paragraph METHODS). Values recorded as zero were less than 1 mμmole.

Amino acid	*Concn. (M)*	*$AT^{32}P$ formed (mμmoles)*	*Amino acid*	*Concn. (M)*	*$AT^{32}P$ formed (mμmoles)*
L-Valine	$2 \cdot 10^{-4}$	61	Threo-β-methyl-DL-aspartic acid	$2.6 \cdot 10^{-2}$	0
L-Threonine	$1.6 \cdot 10^{-2}$	17	L-Penicillamine	$1.6 \cdot 10^{-2}$	0
DL-Allothreonine	$3.2 \cdot 10^{-2}$	0	α-Aminoisobutyric acid	$1.6 \cdot 10^{-2}$	0
L-α-Aminobutyric acid	$4 \cdot 10^{-3}$	19	DL-Homoserine	$3.2 \cdot 10^{-2}$	0
L-Isoleucine	$4.8 \cdot 10^{-2}$	0	DL-Homocysteine	$3.2 \cdot 10^{-2}$	0
DL-Alloisoleucine	$3.2 \cdot 10^{-2}$	0	L-Alanine	$1.6 \cdot 10^{-2}$	0
L-*tert.*-Leucine	$1.6 \cdot 10^{-2}$	0	DL-Valinol	$1.7 \cdot 10^{-3}$	0
L-Leucine	$1.6 \cdot 10^{-2}$	0	DL-α-Amino-β-methylpropyl phosphonic acid	$1.6 \cdot 10^{-2}$	0
DL-Norvaline	$3.2 \cdot 10^{-2}$	0			
DL-Isovaline	$3.2 \cdot 10^{-2}$	0	DL-α-Aminobutyl phosphonic acid	$1.6 \cdot 10^{-2}$	0

TABLE III

EFFECT OF *N*-ETHYLMALEIMIDE ON VALYLHYDROXAMATE FORMATION

The reaction mixtures consisted of enzyme (83 μg), [^{14}C]valine (0.2 μmole, specific activity 1.25 μC/μmole), potassium phosphate (pH 7.0, 0.8 μmole), $MgCl_2$ (0.75 μmole), ATP (0.75 μmole), salt-free NH_2OH (pH 7.0, 360 μmoles) and *N*-ethylmaleimide (0.21 μmole) in a final vol. of 0.16 ml. After incubation at 37° for 30 min, the formation of [^{14}C]valylhydroxamate was determined as described under EXPERIMENTAL (paragraph METHODS).

N-Ethylmaleimide	*Valylhydroxamate formed (mμmoles/h)*
Present	50
Absent	216
Present*	188
Absent*	186

* The enzyme was preincubated with ATP, $MgCl_2$ and L-valine (vol. 0.075 ml) for 5 min at 37°; the other components were then added.

Substrate specificity. The activity of the enzyme toward a number of amino acids was determined using the ^{32}PPi–ATP exchange assay. In agreement with the studies of BERGMANN, BERG AND DIECKMANN[7], activity was observed with L-threonine and L-α-aminobutyrate. However, a number of structurally related and other amino acids were inactive (Table II).

Effect of sulfhydryl reagents. The formation of valylhydroxamate was markedly inhibited by 1.3 mM *N*-ethylmaleimide (Table III). It is of interest that after preincubation of the enzyme with L-valine, ATP, and $MgCl_2$ no inhibition by this concentration of *N*-ethylmaleimide was observed. Enzyme activity was increased by addition of 2-mercaptoethanol and this reagent reduced the marked inhibition produced by *p*-chloromercuribenzoate (Table IV). Inhibition by the latter reagent was prevented or reduced by preincubation of the enzyme with L-valine, ATP, and $MgCl_2$ (Table V). Inhibition by *p*-chloromercuribenzoate (PCMB) was virtually complete at a concn. of $6 \cdot 10^{-6}$ M; half-maximal inhibition was observed with $1.8 \cdot 10^{-6}$ M PCMB

TABLE IV

EFFECT OF 2-MERCAPTOETHANOL AND PCMB ON VALYLHYDROXAMATE FORMATION

The reaction mixtures contained salt-free NH_2OH (pH 7.0, 450 μmoles), ATP (0.75 μmole), $MgCl_2$ (0.75 μmole), L-[^{14}C]valine (0.2 μmole, specific activity 1.25 μC/μmole), enzyme (40 μg), potassium phosphate (0.8 μmole, pH 7.0), PCMB (0.4 mμmole), 2-mercaptoethanol (0.7 μmole) in a final vol. of 0.2 ml; incubated at 37° for 30 min.

*Preincubation conditions**	*Valylhydroxamate formed (mμmoles/h)*
No additions	63
2-Mercaptoethanol	104
PCMB	30
PCMB; 2-mercapto-ethanol**	98

* The enzyme was preincubated for 5 min at 26° in a vol. of 0.155 ml.
** Two successive preincubations (0.155 ml; 0.165 ml).

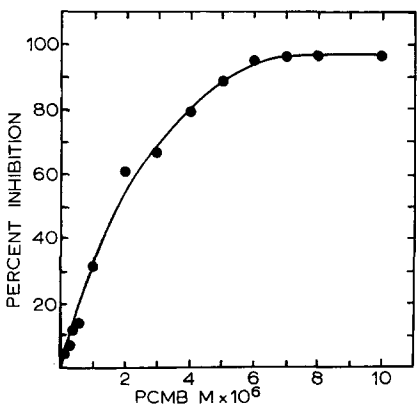
TABLE V

EFFECT OF PREINCUBATION ON INHIBITION BY PCMB

The reaction mixtures contained salt-free NH_2OH (pH 7.0, 450 μmoles), ATP (0.75 μmole), $MgCl_2$ (0.75 μmole), L-[^{14}C]valine (0.2 μmole, specific activity 1.25 μC/μmole), potassium phosphate (pH 7.0, 0.6 μmole), PCMB (0.4 mμmole), and enzyme (70 μg) in a final vol. of 0.20 ml. When L-valine, ATP, and Mg^{2+} were added during the 5-min preincubation period the reaction mixtures were otherwise complete (vol., 0.155 ml) except for PCMB and NH_2OH; after addition of NH_2OH, the mixtures were incubated for 30 min at 37°.

Conditions of preincubation	*PCMB*	*Valylhydroxamate formed (mμmoles/h)*
L-Valine, ATP, Mg^{2+} (0°)	Absent	60
L-Valine, ATP, Mg^{2+} (0°)	Present	57
— (0°)	Present	28
L-Valine, ATP, Mg^{2+} (37°)	Absent	60
L-Valine, ATP, Mg^{2+} (37°)	Present	40
— (37°)	Present	13

(Fig. 4). Titration of the enzyme sulfhydryl groups with PCMB by the procedure of BOYER[12] proceeded relatively slowly with time (Fig. 5). Thus, titration of the enzyme was complete after about 8–10 h; on the other hand, the complete titration took about 10 min when carried out in 8 M urea. The total number of moles of PCMB that reacted with 100 000 g of enzyme under these conditions are given in Table VI. These data indicate that about half of the total enzyme sulfhydryl groups do not react with the reagent in the presence of L-valine, ATP, and $MgCl_2$. In the experiment described in Fig. 4



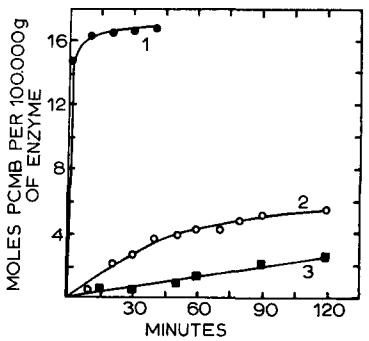


Fig. 4. Effect of PCMB on valylhydroxamate formation. The reaction mixtures contained salt-free NH_2OH (pH 7.0, 450 μmoles), ATP (0.75 μmole), $MgCl_2$ (0.75 μmole), L-[^{14}C]valine (0.2 μmole, specific activity 1.25 μC/μmole), potassium phosphate (1.4 μmole, pH 7.0), enzyme (22 μg) and PCMB as indicated in a final vol. of 0.2 ml; incubated at 37° for 30 min.

Fig. 5. Rate of reaction of PCMB with the enzyme. The reaction mixtures contained enzyme (100–200 μg), potassium phosphate (pH 7.0, 60 μmoles), 2-mercaptoethanol (0.2–0.3 mμmole) and PCMB (0.2 μmole) in a final vol. of 1.4 ml. In Expt. 1, urea (9.6 mmoles) was also present, and in Expt. 3 the reaction mixture also contained ATP (6.6 mμmoles), $MgCl_2$ (2.5 mμmoles), and L-valine (4 mμmoles). The value for the instantaneous reaction of the 2-mercaptoethanol present in the enzyme solution was subtracted in each case.

TABLE VI

TITRATION OF THE ENZYME WITH PCMB

Enzyme (50–70 μg, 1.2 ml) in 0.05 M potassium phosphate (pH 8.0) was titrated by adding 0.02-ml portions of 10^{-4} M PCMB. After each addition the solution was allowed to stand at 26° until the absorbance at 250 mμ was constant. The values given in the table represent the point at which no further absorbance change occurred. Other components of the solution are given in the table. The concentrations of L-valine, ATP, and $MgCl_2$ were, respectively, 1.67, 6.67, and $3.33 \cdot 10^{-6}$ M. Similar values were obtained in experiments in which the enzyme sulfhydryl groups were calculated on the basis of the absorbance changes observed and the extinction coefficient of the PCMB–glutathione complex[12].

Additions	*PCMB/100 000 g of enzyme*
None	16
8 M urea	17
L-Valine, ATP, $MgCl_2$	8

about 95% inhibition was observed with a molar concentration of inhibitor that is about 6 times that of the enzyme (assuming a mol. wt. of 100 000). Since the rate of reaction of the enzyme with PCMB is relatively slow in the presence of L-valine, ATP, and $MgCl_2$ and since there are about 16 sulfhydryl groups per 100 000 g of enzyme, the findings indicate that virtually all activity can be destroyed when a relatively small proportion of the enzyme sulfhydryl groups are blocked.

DISCUSSION

The present method yields a preparation of valyl sRNA synthetase which appears to be more highly purified than others thus far obtained from this source, and preliminary ultracentrifugal and electrophoretic data indicate that the enzyme is homogeneous. The available data indicate that the purified enzyme is stable on storage for at least 4 months. The preparation should therefore be useful for detailed mechanism and structural studies. The general catalytic properties of the present enzyme preparation are, in general, similar to those of previously reported preparations[4,7,8]. The observation that the highly purified enzyme utilizes both threonine and α-aminobutyric acid supports the conclusion that the activities observed toward these amino acids are not due to contamination with other enzymes. It is of note that slight but definite activation was observed with added Zn^{2+} and Co^{2+}; however, the purified enzyme showed some activity in the absence of added metal ion suggesting the presence of some metal ions in the preparation.

Although the enzyme is, like many other aminoacyl sRNA synthetases[13], sensitive to sulfhydryl reagents, the findings indicate that the enzyme-sulfhydryl groups react relatively slowly with PCMB. There are about 16 sulfhydryl groups per 100 000 g of enzyme and approx. half of these do not react with PCMB in the presence of ATP, Mg^{2+}, and L-valine. The present findings also indicate that almost all enzyme activity can be destroyed when only a small proportion of the enzyme-sulfhydryl groups are destroyed. STERN *et al.*[14] also reported that valyl sRNA synthetase of *E. coli* as well as a variety of other aminoacyl sRNA synthetases obtained from this organism are markedly inhibited by PCMB. It appears from their findings that, with the exception

of lysyl sRNA synthetase, all of the aminoacyl RNA synthetases of *E. coli* are sulfhydryl enzymes. It has been reported that about half of the sulfhydryl groups of beef-pancreas tryptophanyl sRNA synthetase are protected by substrates against inhibition by 5,5'-dithiobis (2-nitrobenzoic acid) and PCMB (ref. 15). Similar findings have been made with firefly luciferase[16]. Although the present studies indicate that certain enzyme-sulfhydryl groups are required for activity and that certain enzyme-sulfhydryl groups can be protected against the action of sulfhydryl reagents by enzyme-bound aminoacyl adenylate, the specific relationship between enzyme sulfhydryl groups and activity remains to be elucidated.

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