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Synthesis and Aminoacyl-tRNA Synthetase Inhibitory Activity of Prolyl Adenylate Analogs

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Two nonhydrolyzable prolyl adenylate analogs, 5′-*O*-[*N*-(L-prolyl)-sulfamoyl]adenosine (L-PSA) and 5′-*O*-[*N*-(D-prolyl)-sulfamoyl]adenosine (D-PSA), were prepared in three steps from 2′,3′-di-*O*-isopropylideneadenosine. Both of these compounds inhibited the *in vitro* activity of *Escherichia coli* and human prolyl-tRNA synthetase (ProRS). The human enzyme used in this study was derived from the carboxy-terminal domain of the multifunctional human *EPRS* gene. The K_i^{ATP} values for L-PSA, determined using the ATP–PP_i exchange assay, are very similar for both synthetases (≈ 1–2 nM). The K_i^{Pro} values, on the other hand, vary approximately seven-fold between the two synthetases (0.6 nM for human and 4.3 nM for *E. coli*). The K_i values measured for the D-PSA analog are much higher (51–470 nM) for all cases examined; however, the same species-specific differences are observed with respect to K_i^{Pro}. These results indicate possible structural differences in or near the active sites of the two enzymes that may be exploited in the future design of compounds that function as species-specific synthetase inhibitors *in vivo*.

Aminoacyl-tRNA synthetases catalyze a two-step reaction that results in the esterification of a specific amino acid onto the terminal adenosine of tRNAs. In the first step, ATP and the amino acid combine to form an enzyme-bound intermediate, known as the aminoacyl adenylate. In the second step, the activated amino acid is transferred to the 3′-adenosine of the cognate tRNA. In recent years, the elucidation of a number of X-ray crystal structures of synthetase–substrate complexes has led to a wealth of information about the structure and function of these enzymes (*1*). The 20 aminoacyl-tRNA synthetases have been divided into two classes of 10 members each (*2*). This class division is based on conserved consensus sequences that form part of a nucleotide binding fold and other structural similarities in the core catalytic domains of these enzymes. Ten class I enzymes all share ''HIGH'' and ''KMSKS'' consensus sequences, as well as a nucleotide binding fold known to be the catalytic center of these enzymes. This ''Rossmann'' fold is clearly seen in all of the crystal structures of class I synthetases published to date (*3–7*). Based on computer alignments, identification

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of class-specific motifs was more difficult for the remaining 10 (class II) synthetases, and it was not until the sequence of *Escherichia coli* prolyl-tRNA synthetase (ProRS) was elucidated that a link was found between two previously unrelated subgroups within this class. Moreover, crystal structures of class II synthetases reveal that they contain a similar catalytic domain whose structure is an antiparallel β sheet, very different from the Rossmann fold found in the class I enzymes (*8–13*).

Much less is known about synthetases from higher eukaryotes than those from their prokaryotic counterparts. Sequence comparisons, however, suggest that the sequences of a given enzyme from prokaryotes and eukaryotes are usually more similar to each other than they are to other synthetases of the same class or subclass from the same species (*14, 15*). Nevertheless, differential effects of inhibitors on the activity of a given enzyme from different species have been described. For example, sodium pseudomonate was shown to inhibit both *E. coli* and rat liver isoleucyl-tRNA synthetase (IleRS), but the K_i for the latter was 8000 times greater than the K_i for the *E. coli* enzyme (*16*). The yeast IleRS enzyme is 10^4 times less sensitive to inhibition by pseudomonic acid A than the *E. coli* enzyme (*17*). A species-specific inhibitor of arginyl-tRNA synthetase has also been described (*18*). *E. coli* and human glycyl-tRNA synthetases display species-specific differences in aminoacylation of acceptor-stem minihelices that is governed by the identity of a single nucleotide (*19*). The recently observed differences in $tRNA^{Tyr}$ recognition between the opportunistic pathogen *Pneumocystis carinii* and *E. coli* augur well for the design of a drug selective for the *P. carinii* tryosyl-tRNA synthetase (*20*). Understanding the effects of specific inhibitors on the function of human synthetases is important in this regard.

Nonhydrolyzable L-amino acid sulfamoyl adenosine substrate analogs have been shown to inhibit at least two class II synthetases, but species-specific inhibition by these compounds has not yet been explored. The L-alanyl analog, 5′-*O*-[*N*-(L-alanyl)-sulfamoyl]adenosine, has been shown to inhibit *E. coli* alanyl-tRNA synthetase (*21*). The corresponding seryl compound strongly inhibits *Thermus thermophilus* seryl-tRNA synthetase, and has been used in a recent X-ray crystallographic structure determination of the enzyme–inhibitor complex (*22*). Detailed analysis of the mechanism and specificity of inhibition by these adenylate analogs, however, has not been carried out. The key structural feature of these nonhydrolyzable aminoacyl adenylate analogs is the replacement of the relatively labile phosphate ester of the natural aminoacyl adenylates with the nearly isosteric, yet hydrolytically more stable sulfamoyl linkage (*21*). In this work, we synthesized both L- and D-prolylsulfamoyladenylate (PSA) analogs and investigated the ability of these compounds to inhibit both *E. coli* and human ProRS *in vitro*. The relatively low degree of sequence homology between bacterial and mammalian ProRS species makes this an attractive system for exploring species-specific inhibition (*23*). In addition to gaining mechanistic information about these synthetases, the availability of nonhydrolyzable substrate analogs specific for these enzymes should facilitate the crystallization of these class II synthetases.

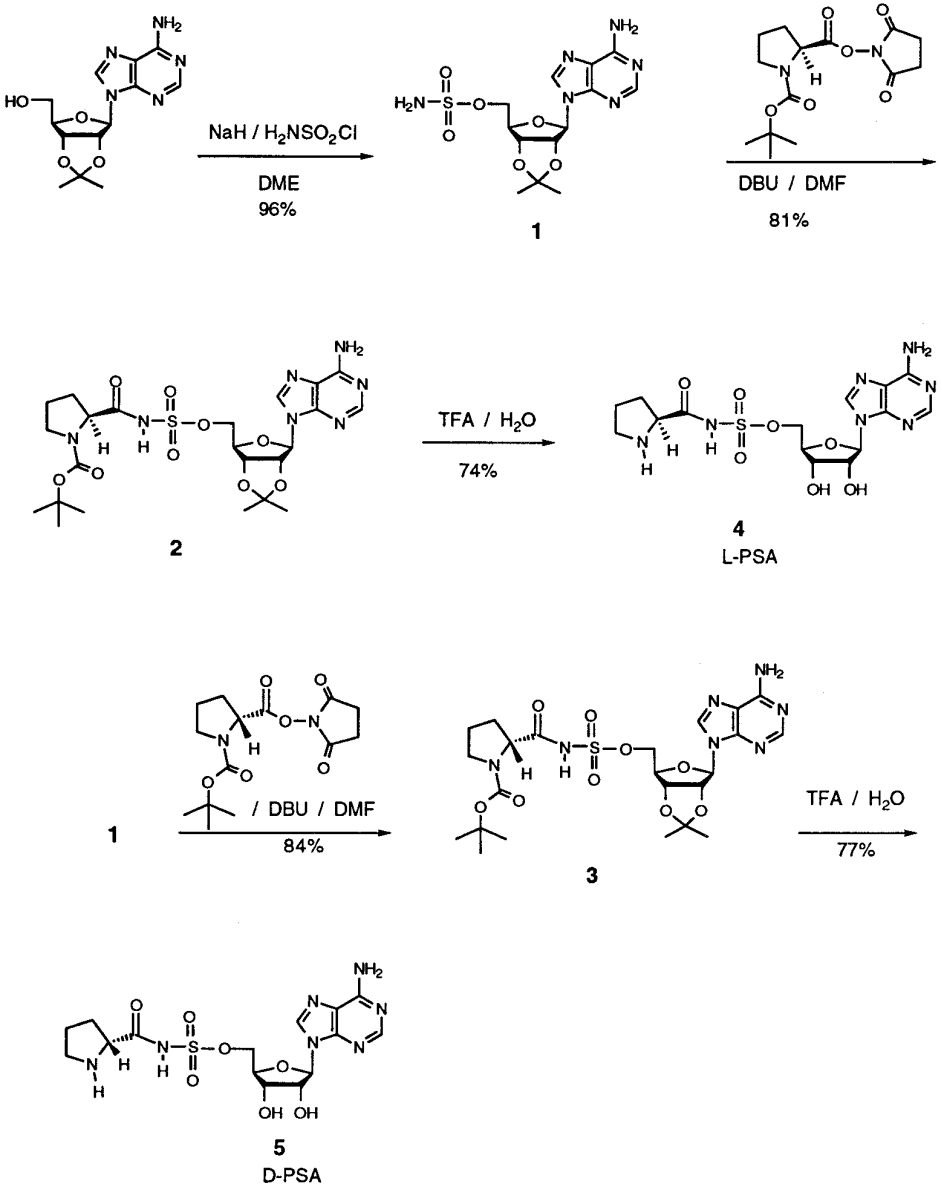
EXPERIMENTAL

Synthesis of Substrate Analogs

All reactions were carried out under an argon or nitrogen atmosphere in oven-dried glassware using standard syringe, cannula, and septa techniques, unless otherwise specified. *N*-t-Boc-L-proline *N*-hydroxysuccinimide ester, *N*-t-Boc-D-proline *N*-hydroxysuccinimide ester, and 2′,3′-*O*-isopropylideneadenosine were purchased from Sigma. Diethyl ether and 1,2-dimethoxyethane (DME) were distilled from Na/benzophenone ketyl under N_2, 1,8-diazabicyclo[5.4]undecane (DBU) and CH_2Cl_2 were distilled from CaH_2, and DMF was vacuum distilled from BaO immediately prior to use. Sulfamoyl chloride was prepared as described (*24*), freshly recrystallized from CH_2Cl_2 at −4°C, and handled only under N_2. Flash chromatography was performed using Baker Flash silica gel 60 (40 μm) and the solvent systems indicated. Analytical and preparative TLC (PTLC) were performed with 0.25 and 0.50-mm EM silica gel 60 F_{254} plates, respectively. NMR spectra are referenced to residual $CHCl_3$ at 7.25 ppm (^{1}H) and 77.0 ppm (^{13}C), DMSO at 2.50 ppm (^{1}H) and 39.5 ppm (^{13}C), or CH_3OH at 3.35 ppm (^{1}H) and 49.0 ppm (^{13}C). Melting points are uncorrected.

2′,3′-O-Isopropylidene-5′-O-sulfamoyladenosine (**1**) (*25, 26*). To a magnetically stirred 0°C solution of 2′,3′-*O*-isopropylideneadenosine (1.00 g, 3.26 mmole) in DME (100 ml) was added NaH (196 mg of a 60% suspension in mineral oil, 4.89 mmol) under N_2. After 30 min at 0°C, a solution of sulfamoyl chloride (565 mg, 4.89 mmol) in DME (30 ml) was added over 15 min. After warming to room temperature and stirring for 16 h, anhydrous methanol (50 ml) was added and the resulting mixture was concentrated to a viscous oil by rotary evaporation. Silica gel chromatography (ethyl acetate–methanol, 10 : 1, v/v) of the residue gave **1** as a white crystalline solid (1.21 g, 3.14 mmol, 96% yield): mp 200–203°C (mp 205–207°C [dec.]) (*25*); R_f 0.12 (ethyl acetate : methanol, 10 : 1, v/v); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.32 (s, 3H), 1.53 (s, 3H), 4.20 (m, 2H), 4.45 (m, 1H), 5.10 (dd, J = 14.8 Hz, 1H), 5.44 (m, 1H), 6.23 (brs, 1H), 7.38 (brs, 2H), 7.66 (brs, 2H), 8.17 (s, 1H), 8.34 (s, 1H); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 25.2, 26.6, 59.2, 81.8, 86.6, 87.2, 93.6, 115.1, 121.3, 140.7, 141.7, 150.5, 158.8. FAB HRMS calcd for $C_{13}H_{19}N_6O_6S$ ([M + H]$^+$): 387.1088. Found: 387.1088.

2′,3′-O-Isopropylidene-5′-O-[N-(Boc-L-prolyl)-sulfamoyl]adenosine (**2**). To a magnetically stirred, room temperature solution of **1** (1.128 g, 2.926 mmol) in DMF (30 ml) was added *N*-t-Boc-L-proline *N*-hydroxysuccinimide ester (1.097 g, 3.51 mmol) and DBU (1.05 ml, 7.02 mmol). After 2 h, TLC showed complete conversion of **2** to a lower-R_f product. The solution was concentrated by rotary evaporation at ca. 3–5 Torr, and the residue was purified by silica gel column chromatography ($CHCl_3$–methanol, 10 : 1, v/v) to give **2** (1.386 g, 2.38 mmol, 81% yield) as a white solid: R_f 0.11 (ethyl acetate–methanol, 10 : 1, v/v); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.31 (s, 9H), 1.42 (s, 3H), 1.57 (s, 3H), 1.8–2.2 (m, 4H), 3.36 (m, 3H), 4.27 (m, 2H), 4.54 (m, 1H), 5.01 (m, 1H), 5.08 (m, 1H), 6.20 (s, 1H), 6.96 (br s, 2H), 8.18 (s, 1H), 8.34 (s, 1H); ^{13}C NMR ($CDCl_3$, 90 MHz): δ 24.3, 25.3, 26.0, 27.1, 28.4, 30.2, 47.0, 62.8, 69.0, 79.6, 81.3, 85.0, 90.8, 114.0, 118.5, 140.4, 149.0, 152.3, 155.0, 175.2, 181.7.



SCHEME 1

2',3'-O-Isopropylidene-5'-O-[*N-(Boc-*D*-prolyl)-sulfamoyl]adenosine* (**3**). This was prepared from **1** (257 mg, 667 μmol), *N*-t-Boc-L-proline *N*-hydroxysuccinimide ester (250 mg, 800 μmol), and DBU (228 μl, 1.6 mmol), in DMF (7 ml), and purified as described for **2** above to provide **3** (327 mg, 561 μmole, 84%) as a white solid:

R_f 0.1 (ethyl acetate–methanol, 10:1, v/v); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.35 (s, 3H), 1.38 (s, 9H), 1.57 (s, 3H), 1.8–2.2 (m, 4H), 3.36 (m, 3H), 4.29 (m, 2H), 4.53 (m, 1H), 5.08 (m, 1H), 5.33 (m, 1H), 6.16 (s, 1H), 7.15 (br s, 2H), 8.09 (s, 1H), 8.25 (s, 1H); ^{13}C NMR ($CDCl_3$, 90 MHz): δ 24.3, 25.4, 27.1, 28.4, 30.6, 47.2, 64.3, 69.0, 80.1, 81.6, 84.9, 91.0, 114.6, 119.5, 149.8, 149.3, 152.9, 155.5, 172.9, 174.1. LR FABMS calcd for $C_{23}H_{34}N_7O_9S$ ($[M + H]^+$): 584.2. Found: 584.2.

5′-O-[(L-*prolyl*)-*sulfamoyl]adenosine* (**4**). A mixture of trifluoroacetic acid and H_2O (8 ml, 5:1, v/v) was added to **2** (680 mg, 1.17 mmol), and the resulting solution was stirred at room temperature for 2 h. The solvents were removed by rotary evaporation, and the residue was suspended in absolute ethanol (20 ml). The ethanol was rotary evaporated, and the residue was resuspended in absolute ethanol (20 ml) and concentrated twice again. The residue was dissolved in 0.1 M aqueous ammonium formate (1 ml) and chromatographed on a column (1 × 24 cm) of Sephadex LH-20 using 70% methanol–H_2O to give **4** (385 mg, 869 mmol, 74%) as a white, amorphous solid: mp 196–199°C (dec.); R_f 0.1 ($CHCl_3$–methanol, 2:1, v/v); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.78–1.9 (m, 3H), 2.15 (m, 1H), 3.2 (m, 2H), 3.92 (m, 1H), 4.05–4.18 (m, 4H), 4.61 (m, 1H), 5.91 (d, J = 5.7 Hz, 1H), 7.30 (br s, 2H), 8.15 (s, 1H), 8.37 (s, 1H). HR FABMS calcd for $C_{15}H_{22}N_7O_7S$ ($[M + H]^+$): 444.1302, found. 444.1323.

5′-O-[(D-*prolyl)-sulfamoyl]adenosine* (**5**). Treatment of **3** (120 mg, 206 μmol) with aqueous trifluoroacetic acid and purification as described above for **4** gave **5** (70 mg, 0.16 mmol, 77%) as a white, amorphous solid: mp 184–188°C (dec.); R_f 0.1 ($CHCl_3$–methanol, 2:1, v/v); ^{1}H NMR (D_2O, 300 MHz): δ 1.9–2.1 (m, 3H), 2.15 (m, 1H), 3.4–3.5 (m, 3H),4.2 (m, 1H), 4.3–4.5 (m, 3H), 4.6 (m, 1H), 6.1 (br d, 1H), 8.31 (s, 1H), 8.37 (s, 1H). LR FABMS calcd for $C_{15}H_{22}N_7O_7S$ ($[M + H]^+$): 444.1302. Found: 444.1308.

Protein Purification

E. coli strain SY327 carrying the plasmids pGT1–2 and pGT1–2# was a gift of Dr. Mike Syvaney. pGT1–2 contains the T7 RNA polymerase gene under the heat-inducible λP_L promoter. pGT1–2# contains the ProRS gene under a T7 polymerase promoter. *E. coli* ProRS purification was performed as described previously except that dithiothreitol and/or 2-mercaptoethanol were not used (*27*). The gene encoding human ProRS was amplified by PCR starting with 1 ng of pZ-3 plasmid DNA (*28*), a gift of Dr. Rolf Knippers, Universität Konstanz, Germany. PCR was performed using primers KY-518 (ccG GAT CCc tca tca agt gga gca gga g) and KY-519 (ccG CTC AGC gtt cat ccc tca gta gct g) at the following temperatures: 94°C 30″–53°C 30″–72°C 90″ (30 cycles). Each primer introduces a *Bam*HI recognition site at the ends of the amplified DNA. The PCR product was digested with *Bam*HI and cloned into the *Bam*HI site of pET-19b (Novagen) to construct pKS509. Plasmid pKS509 produces the ProRS portion (codon 926–1440) of the human GluProRS fusion protein. The expressed protein also has a vector-derived histidine tag and the enterokinase recognition sequence (DDDDK) at the N terminus. The plasmid was transformed into *E. coli* BL21(DE3). Overexpression of human ProRS was induced by addition of 0.1 mM isopropyl β-D-thiogalactopyranoside (IPTG) and cells were

grown at 30°C for 4 h. Phenylmethylsulfonyl fluoride (1 μg/ml, PMSF) was added to the warm medium and cells were harvested by centrifugation and stored at −80°C. All subsequent purification steps were performed at 4°C. The cells were resuspended slowly in 40 ml of sonication buffer (100 mM KH_2PO_4, pH 7.8, 0.3 M NaCl, 2 mM 2-mercaptoethanol, 1 mM PMSF, 0.16 mg/ml benzamidine, 4 μg/ml pepstatin A, 4 μg/ml leupeptin). Disruption of cells was achieved by mild sonication. Cell debris was removed by centrifugation for 45 min at 15,000 *g*. Protamine sulfate (0.5 mg/ml) was added to the supernatant and stirred slowly for 20 min. The precipitated DNA was removed by centrifugation for 20 min at 15,000 *g*. The supernatant was loaded directly onto a 3-ml column containing nickel(II) chelated to a nitrilotriacetic acid-modified Sepharose resin (Ni-NTA, Qiagen), equilibrated with 30 ml sonication buffer, at ≈3 ml/h/resin. Fifty milliliters of wash buffer (100 mM KH_2PO_4, pH 6.0, 0.3 M NaCl, 2 mM 2-mercaptoethanol, 10% glycerol) was applied to the column followed by 15 ml of 0.2 M imidazole in the same buffer. The protein was eluted with 5-ml aliquots of 0.5 M imidazole in wash buffer. Protein purity was greater than 95% as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Purified human ProRS (≈2 mg/ml) was stored at −80°C in 50 mM Hepes (pH 7.5), 2 mM 2-mercaptoethanol, 45% glycerol. Total protein concentrations were determined by the method of Bradford using the Bio-Rad protein assay kit and bovine serum albumin (BSA) as the standard (*29*). This assay was calibrated for ProRS by quantitative amino acid analysis performed in the Microchemical Facility at the Institute of Human Genetics of the University of Minnesota (*30*). Comparison of the results obtained by both methods, using aliquots of the same sample, reveals that the Bradford method overestimates the ProRS protein concentration by 44% for *E. coli* ProRS and 29% for human ProRS. The active-site concentrations of both enzymes determined by the method of Fersht *et al.* (*31*) were used in all kinetic experiments, and we assumed the presence of two active sites per homodimer.

tRNA Preparation

Unmodified *E. coli* $tRNA^{Pro}$ was prepared by *in vitro* transcription as described previously (*27*). This tRNA lacks a cytidine nucleotide at the first position, but is fully functional in *in vitro* aminoacylation assays (*27, 32*). The unmodified human $tRNA^{Pro}$ was prepared using a similar strategy. Briefly, the gene for the human $tRNA^{Pro}$ UGG isoacceptor (*33*) was assembled by cloning a set of six overlapping synthetic DNA oligonucleotides into the *Eco*RI and *Bam*HI sites of pUC119 (*34*). The upstream consensus promoter sequence of T7 RNA polymerase and a downstream *Bst*NI restriction site were also introduced into the plasmid. *Bst*NI linearized DNA was then used to prepare *in vitro* runoff full length human $tRNA^{Pro}$ transcripts using established procedures (*35, 36*). RNA transcripts were purified on denaturing 12% polyacrylamide gels. The tRNA was renatured by heating at 80°C for 3 min in 0.1 M Tris-Cl, pH 8, 1 mM EDTA. The mixture was transferred to a 60°C water bath for 3 min. $MgCl_2$ was added to 10 mM and the mixture cooled to room temperature over several minutes and finally placed on ice. The concentration was determined using an extinction coefficient of $\varepsilon_{260} = 6.04 \times 10^5\ M^{-1}$. Stock solutions were stored at −20°C.

Dithionitrobenzoic Acid Modification of E. coli ProRS

Dithionitrobenzoic acid (DTNB) modification was accomplished using a 13:1 probe to *E. coli* ProRS ratio in 100 mM TrisCl, pH 8.0. A 2 mM DTNB stock solution was prepared immediately prior to use in 100 mM TrisCl, pH 8.0, 1 mM EDTA, and kept in the dark. G-50 Sephadex (Pharmacia) spin columns were equilibrated with 100 mM TrisCl, pH 8.0, such that the packed resin volume was 1.5 ml, and kept at 4°C until use. Three 120-μl reaction mixtures containing 37.5 μM ProRS and L-PSA at 0, 37.5, and 375 μM in 100 mM TrisCl, pH 8.0, were incubated at 22°C for 10 min. A control reaction without ProRS was prepared similarly to determine background corrections. The labeling reactions were initiated by addition of 30 μl 2 mM DTNB to a final concentration of 400 μM and kept in the dark. After 60 and 120 min, the reaction mixtures were applied to the spin columns and centrifuged at 570*g* for 3 min to remove unreacted DTNB. The recovered enzyme solutions were denatured with 6 M guanidine–HCl and the total protein concentrations determined by the method of Bradford. DTNB was released from the enzyme by incubation of the mixtures with 5 mM DTT for 20 min at 22°C. The release of 5-mercapto-2-nitrobenzoate (TNB) was monitored at 412 nm (ε = 13.7 mM^{-1}) (*37*).

Active-Site Titration

Determination of the active-site concentrations of *E. coli* and human ProRS was carried out in triplicate essentially as described (*31*). Briefly, the standard reaction mixture (133 μl) contained 144 mM TrisCl pH 8.0, 5.0 μM [γ-^{32}P]ATP ($\approx$10 μCi/ml), 1 mM proline, 10 mM $MgCl_2$, and 5 U/ml pyrophosphatase at 22°C. Prior to initiation of the reaction with enzyme, three 10-μl aliquots were removed from the reaction mixture and put into 1 ml of stop solution (11% $HClO_4$, 1% activated charcoal). The reaction was then initiated by addition of $\approx$2.25 μM ProRS as determined using the Bradford method described above (*29*). Nine 10-μl aliquots were then quenched in stop solution at various times up to 12 min. The quenched solutions were vacuum filtered on glass circles (2.4-cm diameter, Schleicher & Schuell No. 06390) and washed twice with 7 ml 1% $HClO_4$ followed by 7 ml of water. The air-dried filters were counted in 5 ml scintillation fluor. The standard deviation was less than 5 % for all determinations.

Aminoacylation Assay

Kinetic parameters were determined for the $tRNA^{Pro}$ transcripts using concentrations that ranged from 1 to 8 μM. The standard reaction mixture (60 μl) contained 50 mM Hepes pH 7.5, 0.2 mg/ml BSA, 20 mM KCl, 25 mM $MgCl_2$, 20 mM 2-mercaptoethanol, 5 mM ATP, and 20 μM [^{3}H]proline (0.3 mCi/ml). Reactions were initiated by addition of 0.05 μM ProRS. Five 10-μl aliquots of the reaction mixtures were quenched over a 2-min period by application to paper filters (2.3-cm diameter, Whatman No. 1030 023) pretreated with 5% trichloroacetic acid. Filters were washed, dried, and counted as described (*38*).

ATP–PP_i Exchange Assay

The ATP–PP_i exchange reaction was performed essentially as described (*39, 40*). Michaelis–Menten kinetic parameters were determined using 2 mM constant substrate (ATP or proline), with the corresponding variable substrate ranging from 0.025 to 5 mM. The standard reaction solutions (645 μl) containing 144 mM TrisCl pH 8.0, 10 mM KF, 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.2 mg/ml BSA, 1.0 mM $^{32}PP_i$ ($\approx$10 μCi/ml), and the appropriate combination of ATP and proline were incubated for 10 min at 37°C. Reactions were initiated by addition of 0.5 nM ProRS. At the desired time intervals, 100-μl aliquots of the reaction mixture were quenched in 1 ml solutions of 11% $HClO_4$, 0.3 M PP_i, and 1% activated charcoal at 4°C. The quenched solutions were vacuum filtered on glass circles (2.4-cm diameter, Schleicher & Schuell No. 06390) and washed with 7 ml 1% $HClO_4$, 25 mM PP_i followed by 7 ml water. The air-dried filters were counted in 5 ml scintillation fluor. The specific activity was determined by counting 10 μl of the reaction mixture injected directly into 5 ml scintillation fluor. For the inhibition studies, 100-μl solutions containing 0.5 nM ProRS were incubated in 144 mM TrisCl, pH 8.0, 0.2 mg/ml BSA, and the appropriate concentrations of inhibitor and variable substrate (ATP or proline) for 10 min at 37°C. Reactions were initiated by addition of 550 μl of 144 mM TrisC,l pH 8.0, 12 mM $MgCl_2$, 12 mM KF, 12 mM 2-mercaptoethanol, 2.4 mM of the constant substrate (ATP or proline), and 1.2 mM $^{32}PP_i$ ($\approx$10 μCi/ml) at 37°C. One hundred-microliter aliquots were removed at the desired times and quenched, filtered, and counted as above. The concentrations of the substrates and inhibitors are given in the table and figures. Initial reaction rates were determined using linear least-squares analysis. The kinetic constants K_M^{Pro}, K_M^{ATP}, and k_{cat} were determined from Lineweaver–Burk plots. Dixon plots were produced and the inhibition constants (K_i) were determined from replots of the slopes versus 1/[S] (*41*), with standard errors calculated as described (*42*).

RESULTS AND DISCUSSION

In higher eukaryotes, prolyl-tRNA synthetase is present as part of a multifunctional enzyme that also displays glutamyl-tRNA synthetase activity and has been referred to as the ''gluprolyl-tRNA synthetase'' (gluproRS) (*43*). This bifunctional protein has been found in *Drosophila* (*43*) rat liver (*44*), sheep liver (*45*), and Hela cells (*23, 28*). In the case of the *Drosophila* enzyme, the amino and carboxy-terminal domains were expressed separately in *E. coli*, and were found to catalyze the aminoacylation of glutamic acid and proline tRNA species, respectively (*43*). The low degree of sequence homology between the carboxy-terminal part of the mammalian proteins and *E. coli* ProRS (22%) (*23*) made the initial assignment of these functions to the human polypeptide problematic (*28, 43*). In this work, we express the carboxy-terminal domain (amino acid residues 926–1440) of the human gluproRS polypeptide in *E. coli*. Our construct also contains an amino-terminal histidine tag to facilitate purification. This portion of the human polypeptide was purified and shown to aminoacylate crude bovine tRNA (Sigma) with [^{3}H]proline (data not