Olins, A. L., Carlson, R. D., Wright, E. B., & Olins, D. E. (1976) *Nucleic Acids Res. 3*, 3271–3291.
Pardon, J. F., Worcester, D. L., Wooley, J. C., Tatchell, K., van Holde, K. E., & Richards, B. M. (1975) *Nucleic Acids Res. 2*, 2163–2176.
Richmond, T. J., Finch, J. T., Rushton, B., Rhodes, D., & Klug, A. (1984) *Nature (London) 311*, 532–537.
Sibbet, G. J., Carpenter, B. G., Ibel, K., May, R. P., Kneale, G. G., Bradbury, E. M., & Baldwin, J. P. (1983) *Eur. J. Biochem. 133*, 393–398.
Stuhrmann, H. B. (1974) *J. Appl. Crystallogr. 7*, 173–178.
Thoma, F., Koller, Th., & Klug, A. (1979) *J. Cell Biol. 83*, 403–427.
Überbacher, E. C., & Bunick, G. J. (1985) *J. Biomol. Struct. Dyn. 2*, 1033–1055.
Überbacher, E. C., Ramakrishnan, V., Olins, D. E., & Bunick, G. J. (1983) *Biochemistry 22*, 4916–4923.
Ueki, T., Hiragi, Y., Kataoka, M., Inoko, Y., Amemiya, Y., Izumi, Y., Tagawa, H., & Muroga, Y. (1985) *Biophys. Chem. 23*, 115–124.
Watanabe, K., & Iso, K. (1981) *J. Mol. Biol. 151*, 143–163.
Wu, H., Dattagupta, N., Hogan, M., & Crothers, D. M. (1979) *Biochemistry 18*, 3960–3965.
Zama, M., Bryan, P. N., Harrington, R. E., Olins, A. L., & Olins, D. E. (1978) *Cold Spring Harbor Symp. Quant. Biol. 42*, 31–41.

Purification and NMR Studies of [*methyl*-^{13}C]Methionine-Labeled Truncated Methionyl-tRNA Synthetase†

Paul R. Rosevear
Department of Biochemistry and Molecular Biology, University of Texas Medical School, Houston, Texas 77225
Received January 13, 1988; Revised Manuscript Received June 9, 1988

ABSTRACT: A procedure for the rapid purification of a truncated form of the *Escherichia coli* methionyl-tRNA synthetase has been developed. With this procedure, final yields of approximately 3 mg of truncated methionyl-tRNA synthetase per gram of cells, carrying the plasmid encoding the gene for the truncated synthetase [Barker, D. G., Ebel, J.-P., Jakes, R., & Bruton, C. J. (1982) *Eur. J. Biochem. 127*, 449], can be obtained. The catalytic properties of the purified truncated synthetase were found to be identical with those of the native dimeric and trypsin-modified methionyl-tRNA synthetases. A rapid procedure for obtaining milligram quantities of the enzyme is necessary before the efficient incorporation of stable isotopes into the synthetase becomes practical for physical studies. With this procedure, truncated methionyl-tRNA synthetase labeled with [*methyl*-^{13}C]methionine was purified from an *Escherichia coli* strain auxotrophic for methionine and containing the plasmid encoding the gene for the truncated methionyl-tRNA synthetase. Both carbon-13 and proton observe–heteronuclear detect NMR experiments were used to observe the ^{13}C-enriched methyl resonances of the 17 methionine residues in the truncated synthetase. In the absence of ligands, 13 of the 17 methionine residues could be resolved by carbon-13 NMR. Titration of the synthetase, monitoring the chemical shifts of resonances B and M (Figure 3), with a number of amino acid ligands and ATP yielded dissociation constants consistent with those derived from binding and kinetic data, indicating active site binding of the ligands under the conditions of the NMR experiment. The maximum chemical shift change of resonance B, in the presence of saturating concentrations of ligands, was found to be dependent on the exact nature of the amino acid side chain. Differences in the magnitude of the substrate-induced conformational change, detected by monitoring resonance B, may be critical in triggering the recognition processes between cognate amino acid and synthetase. In contrast, the chemical shift of resonance M was found to be dependent on the negative charge introduced by the ligands at the aminoacyl adenylate site. This correlation is consistent with an induced-fit mechanism where portions of the binding site are formed as the various ligands are introduced into the aminoacyl adenylate site. Further studies on the solution structure of synthetase–ligand complexes will be useful in probing the structural mechanisms used in catalysis and amino acid discrimination.

The methionyl-tRNA synthetase is a member of a family of enzymes responsible for ligation of specific amino acids to their cognate tRNAs (Schimmel & Soll, 1979). This reaction occurs in two separate steps, and the specificity exhibited toward the substrates in each step is essential in maintaining the fidelity of information transfer from DNA to protein. The *Escherichia coli* methionyl-tRNA synthetase is a dimeric zinc metalloprotein with an apparent subunit molecular mass of 76 000 daltons (Barker et al., 1982). Two ATP binding sites per subunit have been found for the native dimeric enzyme (Fayat & Waller, 1974). The role of the second ATP binding site is unknown. Mild proteolysis with trypsin is known to produce a fully active monomeric fragment with an apparent molecular mass of 64 000 daltons (Cassio & Waller, 1974). The active trypsin fragment is produced by removal of about 130 amino acid residues from the carboxy terminus of the native protein. Kinetic properties of the trypsin fragment were found to be identical with those of the native dimeric enzyme (Blanquet et al., 1974; Hayafil et al., 1976). However, the monomeric enzyme was found to have only a single ATP binding site, it being the catalytic site (Fayat & Waller, 1974).

† This work was supported by a grant from the Robert A. Welch Foundation, AU-1025.

0006-2960/88/0427-7931$01.50/0

Recently, a truncated form of the native *E. coli* methionyl-tRNA synthetase has been cloned and found to complement a methionine auxotroph in which the lesion is a defective methionyl-tRNA synthetase (Barker et al., 1982). The truncated methionyl-tRNA synthetase (ΔMTS)[1] was found to have a molecular mass of 66 000 daltons and represents a slightly larger polypeptide than the trypsin fragment (Barker et al., 1982).

The three-dimensional structure of the trypsinized form of the methionyl-tRNA synthetase with ATP bound has been resolved at 2.5 Å (Brunie et al., 1987). The protein is organized into two structural domains with the N-terminal domain containing enzyme-bound zinc and a nucleotide binding fold as part of the aminoacyl adenylate site. The enzyme-bound zinc is located near the β-phosphate of the bound ATP and is known to have a structural role in maintaining an active conformation of the enzyme (Brunie et al., 1987). However, it is unclear if the enzymic zinc also plays a role in catalysis or substrate recognition.

Fluorescence spectroscopy has been used to monitor couplings, changes in the dissociation constant of one ligand upon the binding of another ligand, between the binding sites for methionine and nucleotide on the trypsin-modified methionyl-tRNA synthetase (Fayat et al., 1977). However, the nature of the substrate-induced conformational changes occurring in the synthetase has not been monitored. Conformational changes occurring at the intersubunit domain of the native dimeric synthetase upon $tRNA^{fMet}$ binding have been observed by use of fluorescent-labeled protein (Ferguson & Yang, 1986). These changes suggested a segmental flexibility in the synthetase that was altered by $tRNA^{fMet}$ binding (Ferguson & Yang, 1986).

As part of a general study to better understand the molecular basis for the substrate specificity exhibited by the methionyl-tRNA synthetase, we have initiated an NMR study of the truncated enzyme and its complexes with cognate and noncognate substrates and analogues. For such studies, it is necessary to resolve resonance signals from individual amino acid residues in the enzyme. To facilitate this on a relatively large protein, we have initially purified milligram quantities of [*methyl*-^{13}C]methionine-enriched synthetase. Conformational changes in the methionyl-tRNA synthetase induced by substrate binding could be followed by monitoring chemical shift changes in the carbon-13-enriched methyl resonances. The magnitude and direction of the chemical shift changes were found to be dependent on the nature of the amino acid side chain and the negative charge donated by the ligands.

Experimental Procedures

Materials. [*methyl*-^{14}C]-L-Methionine and [^{32}P]pyrophosphate were purchased from New England Nuclear. [*methyl*-^{13}C]-L-Methionine and DEAE-Sepharose were obtained from Cambridge Isotope Labs and Pharmacia Chemicals, respectively. Crude tRNA was either purchased from Boeringer-Manhiem or prepared from *E. coli* by phenol and 2-propanol precipitation. All other chemicals were of the highest purity available commercially.

Enzyme Assays. The methionine-dependent ATP–pyrophosphate exchange assay was performed as described earlier (Heinrikson & Hartley, 1967; Fersht & Dingwall, 1979). The rate of methionyl-tRNA formation was assayed according to Barker et al. (1982) at 30 °C. Prior to the reactions, stock solutions of the enzyme were appropriately diluted in standard buffer containing 0.1 mg/mL BSA. Protein concentration was estimated by Bradford (1976) or from the known extinction coefficients at 280 nm (Blanquet et al., 1973). Active site titration by filtration through nitrocellulose disks was used to determine the concentration of the purified synthetase (Hyafil et al., 1976; Mulvey & Fersht, 1976).

Bacterial Strains and Growth. The bacterial strain MJ1000D carrying the plasmid MTS-a5 overproducing the *E. coli* truncated methionyl-tRNA synthetase was obtained from Dr. David G. Barker. This strain was produced by transforming MJ1000D with pBR322/EcoMTS-a5 (Barker et al., 1982). MJ1000D is auxotrophic for methionine, having a chromosomal methionyl-tRNA synthetase with a K_m for methionine approximately 350-fold higher than that of the wild-type enzyme (Rigby et al., 1976). Growth of MTS-a5 was routinely performed in M9 media (7 g of Na_2HPO_4, 3 g of KH_2PO_4, 1 g of NH_4Cl, 0.5 g of NaCl, 10 mL of 20% glucose, 1 mL of 1% thiamin, 0.1 mL of 1 M $CaCl_2$, 1 mL of 1 M $MgSO_4$, and 0.3 mL of 0.01 M $FeCl_3$ per liter) supplemented with 40 μg/mL ampicillin at 37 °C. Before large-scale growth, aminoacylation assays were performed on an aliquot from several individual colonies. The specific activity of ΔMTS in these crude extracts varied from 120 to 410 nmol min^{-1} mg^{-1}. The culture with the highest specific activity was used in the large-scale (188-L) fermentation. The large-scale fermentation was allowed to proceed for 12 h before the cells were harvested in a prechilled, continuous-flow centrifuge. The yield was usually 2 g of cell paste per liter of culture. An equal weight of cells and 20 mM Tris buffer containing 10% sucrose at pH 7.5 were mixed, poured into liquid nitrogen, and stored at −70 °C. Cells grown and harvested as described above were routinely used for the purification of ΔMTS.

Incorporation of [methyl-^{13}C]Methionine into the Truncated Methionyl-tRNA Synthetase. [*methyl*-^{13}C]-Methionine-enriched methionyl-tRNA synthetase was prepared by growing an *E. coli* strain auxotrophic for methionine and transformed with pBR322/MTS-a5 in minimal media supplemented with [*methyl*-^{13}C]-L-methionine. In preliminary experiments cell growth was monitored as a function of added methionine. Although the percent incorporation of methionine from the media into protein was not measured, it was determined that the yield of cells per liter of culture and the specific activity of the synthetase in the crude extract were greatest at 20 μg/mL added methionine. Cell growth was performed as described above except the large-scale (125-L) fermentation was supplemented with 20 μg/mL [*methyl*-^{13}C]-L-methionine. Yields of cells were similar to those obtained in unlabeled cell growths.

Buffers. Cell lysis buffer contained 50 mM Tris (from 1 M Tris, pH 8.0), 5% glycerol, 2 mM EDTA, 1 mM DTT, 0.24 M NaCl, 1.4 mM mercaptoethanol, and 0.1 mM PMSF. TGED buffer is 10 mM Tris (from 1 M Tris, pH 8.0), 5% glycerol, 0.1 mM EDTA, 0.2 M NaCl, 0.1 mM PMSF, and 1 mM DTT. Buffer A contained 50 mM Tris-HCl, 10 mM mercaptoethanol, and 0.1 mM PMSF at pH 7.5. Buffer B contained 10 mM potassium phosphate, 10 mM mercaptoethanol, and 0.1 mM PMSF at pH 6.8.

Purification of the Truncated Methionyl-tRNA Synthetase. The following procedure is for 250 g of MJ1000D cells transformed with pBR322/EcoMTS-a5. The basic steps consist of cell lysis, Polymin P precipitation, ammonium sulfate

[1] Abbreviations: ΔMTS, truncated methionyl-tRNA synthetase; DTT, 1,4-dithiothreitol; BSA, bovine serum albumin; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; Tris, tris(hydroxymethyl)aminomethane; DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate; HPLC, high-pressure liquid chromatography.

Table I: Purification of the Truncated Methionyl-tRNA Synthetase

fraction	total activity ($\times 10^6$ units)	total protein (mg)	sp act. (units)	x-fold purification	% recovery
(I) cell-free lysate	1.75	5300	300		100
(II) Polymin P precipitation	1.74	2700	600	2	99
(III) ammonium sulfate precipitation	1.72	1200	1400	5	98
(IV) DEAE-Sepharose	1.40	400	3500	12	64

precipitation, and DEAE-Sepharose chromatography. All steps are carried out at 4 °C unless noted otherwise.

(I) Cell Lysis. Frozen cells, 250 g, are stirred at room temperature in 550 mL of lysis buffer until homogeneous. At 4 °C, 250 mg of lysozyme is added and the suspension stirred for 30 min. Freshly made 4% sodium deoxycholate is added to a final concentration of 0.5%; the solution is stirred for an additional 2 min and incubated for an additional 25 min. To this solution, 550 mL of TGED buffer is added, the viscous solution is stirred for 5 min, and 1 L of buffer A is added with stirring. The chromosomal DNA is sheared in a blender at medium speed for 30 s. The cell lysate is then centrifuged at 7000*g* for 1 h. The supernatant is fraction I (2660 mL).

(II) Polymin P Precipitation. Polymin P is added dropwise to fraction I with constant stirring to a final concentration of 0.2%; the solution is stirred for an additional 15 min and centrifuged at 7000*g* for 20 min. The precipitate is discarded and the supernatant, fraction II (2710 mL), used in the next step.

(III) Ammonium Sulfate Precipitation. Solid ammonium sulfate (668 g) is slowly added to fraction II with constant stirring over a 30-min period to 35% saturation. After complete addition of the ammonium sulfate, the suspension is stirred for an additional 30 min. The ammonium sulfate precipitate is collected by centrifugation at 7000*g* for 45 min and the supernatant made 55% saturated in ammonium sulfate by the addition of 475 g of solid ammonium sulfate. The suspension is stirred an additional 30 min and the precipitate collected by centrifugation at 7000*g* for 60 min. The supernatant is discarded. The precipitate is dissolved in 100 mL of buffer B and dialyzed overnight against 6 L of buffer B to give fraction III (350 mL).

(IV) DEAE-Sepharose Chromatography. Fraction III, 350 mL, is centrifuged at 7000*g* for 15 min and loaded onto a 3 × 45 cm DEAE-Sepharose fast-flow column equilibrated in buffer B. After fraction III is loaded onto the column, the column is washed with 1 L of buffer B. ΔMTS is eluted with a 2.0-L linear gradient from 0 to 0.3 M NaCl in buffer B. The synthetase elutes at approximately 0.2 M NaCl with a specific activity in the aminoacylation assay of 3500 units/mg. An SDS–polyacrylamide gel of the pooled fraction from the DEAE-Sepharose column revealed one major band (>95%) corresponding to ΔMTS (Figure 1). The purified synthetase is made 50% in glycerol and stored at −20 °C.

Enzyme Preparation for NMR Studies. Before use in NMR experiments, ΔMTS, stored in 50% glycerol, was diluted 10-fold with buffer B and dialyzed overnight in buffer B. Concentration and deuteriation of ΔMTS were accomplished by pressure filtration in an Amicon cell using a PM10 membrane. All buffer and reagent solutions used in the NMR studies were treated with Chelex 100 before use to remove trace metal contaminants. After NMR experiments, which occasionally lasted up to 24 h, ΔMTS was found to retain at least 90% of its original activity.

Magnetic Resonance Methods. Carbon-13 NMR spectra were obtained on a GE 500 MHz NMR operating at 125.7 MHz for carbon. Spectra were recorded with 1024 transients, each with a 20-kHz spectral width, 16K data points, a 60° pulse, a 3-s repetition time, and broad-band proton decoupling with WALTZ-16 modulation (Shaka et al., 1983) at 25 °C. When necessary, resolution was enhanced by apodization of the FID by Gaussian multiplication and an exponential multiplication with a negative time constant. Carbon chemical shifts are reported relative to internal dioxane at 67.4 ppm. A lock was provided by the addition of 10% D_2O to the sample.

^1H NMR spectra were recorded at 500 MHz. Proton-detected heteronuclear edited spectra were obtained with the echo difference pulse sequence described by Griffey et al. (1985). The value of the delay (Δ) which selects the ^{13}C-coupled proton phase in the echo was set at 3.4 ms. Spectra were obtained with a 10000-Hz spectral width, 8K data points, a 1.5-s repetition time, a 90° ^1H pulse of 8.8 μs, and a 90° ^{13}C pulse of 28 μs at 25 °C. Two-dimensional forbidden echo spectra were obtained by the method of Bax et al. (1983). Proton chemical shifts are reported relative to external DSS.

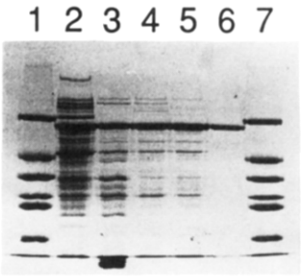

FIGURE 1: The 10% SDS–polyacrylamide gel electrophoresis monitoring the purification of ΔMTS from the *E. coli* strain MJ1000D carrying the plasmid, MTS-a5, overproducing the *E. coli* truncated methionyl-tRNA synthetase. Lanes 1 and 7, molecular mass standards [bovine serum albumin (68 kDa), hen egg albumin (45 kDa), carbonic anhydrase (29 kDa), soybean trypsinogen (24 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.2 kDa)]; lane 2, cell free lysate; lane 3, supernatant from 0.2% Polymin P precipitation of lane 4, dialyzed fraction from the 35–55% ammonium sulfate cut; lane 5, heat treatment at 50 °C for 20 min; lane 6, ΔMTS eluted from DEAE-Sepharose column. The heat treatment, lane 5, was not routinely used in the purification of ΔMTS. The gel was stained with Coomassie Brilliant Blue.

RESULTS

Expression and Purification of the Truncated Methionyl-tRNA Synthetase. Growth of *E. coli* MJ1000D transformed with the plasmid pBR322/EcoMTS-a5 was performed in minimal media to increase the selective pressure for production of ΔMTS. The parent strain MJ1000D is unable to grow in minimal media, due to a mutant chromosomal synthetase, without the addition of 40 μg/mL methionine (Rigby et al., 1976; Barker et al., 1982). Consistently higher specific activities for ΔMTS were observed from cells grown in minimal media as compared to enriched media. Aminoacylation activities performed on crude extracts showed a 100-fold overproduction of MTS in cells carrying the plasmid pBR322/EcoMTS-a5 similar to that reported by Barker et al. (1982).

The purification procedure is summarized in Table I, and the SDS–polyacrylamide gel electrophoresis monitoring the purification from crude extracts is shown in Figure 1. The overall purification from 125 g of cell paste (250 g of 1:1 mixture of cells and Tris/sucose buffer) is 400 mg of appar-

Table II: Comparison of the Various Forms of the *E. coli* Methionyl-tRNA Synthetase

	native	truncated	trypsin fragment
sp act.			
aminoacylation[a]	2000[c]	3500	3500[c]/2800[c]
ATP–PP exchange[b]	1200[c]	1200	1100[c]/1200[d]
K_m (μM)[e]			
ATP	370[c]	200	430[c]/380[d]
methionine	40[c]	40	40/nd
native molecular mass (daltons)	152000[f]	66000[g]	64000[f]
no. of subunits	2	1	1

[a] Units of enzyme activity expressed as nmol min^{-1} mg^{-1} under standard assay conditions at 30 °C. [b] Units of enzyme activity expressed as μmol (15 min)$^{-1}$ mg^{-1} under standard assay conditions at 37 °C. [c] From Cassio and Waller (1971a,b). [d] Trypsin fragment obtained from treatment of purified truncated methionyl-tRNA synthetase with trypsin. [e] Values measured in the ATP–pyrophosphate exchange assay as described under Experimental Procedures. [f] Molecular mass determined by Kock and Burton (1974). [g] Molecular mass of the native truncated methionyl-tRNA synthetase determined by HPLC gel filtration, under the conditions described in Figure 2.

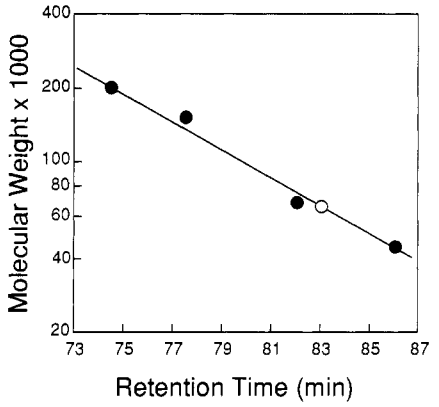

FIGURE 2: Estimation of the native molecular mass of the purified truncated methionyl-tRNA synthetase. Gel filtration was performed on a Superose 12 HPLC column in 20 mM Tris-HCl buffer, pH 7.6, containing 50 mm NaCl, 0.1 mM EDTA, and 0.1 mM DTT. A calibration curve was prepared with the following molecular mass standards (●): β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (68 kDa), and chicken egg albumin (45 kDa). The truncated methionyl-tRNA synthetase (○) eluted at a position corresponding to a molecular mass of 66 kDa.

ently homogeneous enzyme, representing a 64% yield. Purified ΔMTS was routinely stored in 50% glycerol at −20 °C, conditions found to be optimal for both the native and trypsin-modified MTS proteins (Blanquet et al., 1974). With this procedure, large amounts of ΔMTS can be purified in less than 5 days. This type of purification scheme is necessary before efficient incorporation and purification of synthetase specifically enriched with stable isotopes becomes practical. As expected from studies on the trypsin-modified enzyme, deletion of a portion of the carboxy-terminal domain had little effect on the pyrophosphate exchange kinetics (Table II). Proteolysis of the truncated enzyme with trypsin produced a fragment which had a relative mobility on SDS–polyacrylamide gels in good agreement with that published for the trypsin enzyme prepared from native MTS (Barker et al., 1982).

The native molecular mass of ΔMTS, under conditions similar to those of the NMR experiment, was determined by HPLC gel filtration on a Superose 12 column (Figure 2). ΔMTS was found to have a molecular mass of approximately 66 kDa, consistent with it being a monomer in solution (Table II, Figure 2). This was expected since proteolytic treatment of native dimeric MTS yields a monomeric enzyme (Cassio & Waller, 1974), and 80% of these residues are also missing

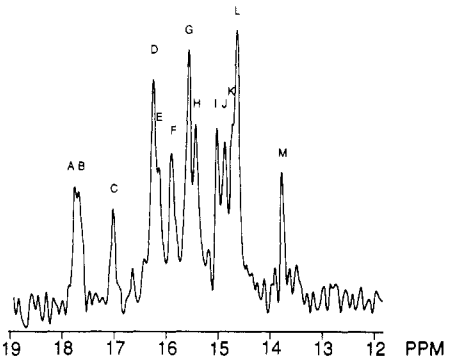

FIGURE 3: The 125.7-MHz ^{13}C NMR spectrum of 0.54 mM [*methyl*-^{13}C]methionine-labeled ΔMTS in 5 mM [$^2H_{11}$]Tris buffer, pH 7.6 containing 50 mM NaCl, 0.1 mM EDTA, and 0.1 mM DTT. Spectra were obtained by collecting 1024 transients, each with broad-band proton decoupling, by use of 16K data points, a spectral width of 20 kHz, a 60° pulse, and a 3-s repetition rate at 25 °C.

from ΔMTS. The addition of saturating concentrations of L-methionine to the HPLC buffer had no effect on the molecular weight of ΔMTS. Gel filtration in buffer B containing 100 mM NaCl also gave a single peak for ΔMTS with a relative mobility consistent with it being a monomer. As expected, gel filtration of the trypsin fragment produced from ΔMTS yielded a single peak with a relative mobility consistent with it having a molecular mass of 64 kDa.

Carbon-13 NMR of [methyl-^{13}C]Methionine-Enriched Truncated Methionyl-tRNA Synthetase. A typical 125.7-MHz spectrum of 0.54 mM [methyl-^{13}C]methionine-labeled ΔMTS is shown in Figure 3. Of the 17 methionine residues in ΔMTS 13 resonances can be resolved between 17.8 and 13.7 ppm and are labeled A through M (Figure 3). Denaturation of ΔMTS with 5 M guanidinium chloride gave a single sharp peak at 16.1 ppm, suggesting that the methionine residues have no constraints on their motion and are in a random coil conformation in the denatured enzyme. The methyl resonances of the 17 methionines exhibit both upfield and downfield shifts from the ^{13}C chemical shift in the guanidinium-denatured protein. The chemical shifts observed for the [methyl-^{13}C]-methionine resonances in ΔMTS are in the range previously reported for the methyl resonances of methionine in other proteins (Eakin et al., 1975; Jones et al., 1976; Blakley et al., 1978; Hines & Chlebowski, 1987). Resonance M exhibits the largest upfield chemical shift, 2.4 ppm, from that observed for methionine in the denatured enzyme. The upfield chemical shift could result from ring current effects of nearby aromatic residues. Resonance A exhibits the largest downfield chemical shift, 1.6 ppm. Electronic effects arsing from a partial positive charge on the methionine sulfur have previously been shown to produce downfield chemical shifts in the methyl resonances of methionine (Blakley et al., 1978). *S*-(Carboxymethyl)-methionine was found to have a chemical shift of 24.3 ppm (Blakley et al., 1978). Edge effects from adjacent aromatic residues may also contribute to the observed downfield shifts. The total chemical shift range observed for the methyl resonances of methionine in ΔMTS is approximately 4 ppm. This range is similar to that previously observed for the methyl resonances in [*methyl*-^{13}C]methionine-labeled dihydrofolate reductase (Blakley et al., 1978).

Selective Observation of the Methionine Methyl Protons in the Truncated Methionyl-tRNA Synthetase. The large one-bond carbon–proton spin–spin coupling constant $^1J_{^{13}C-^1H}$ = 140 Hz was used to selectively observe the methionine methyl protons. Figure 4A shows the ^1H NMR spectrum of 1.3 mM [*methyl*-^{13}C]methionine-labeled ΔMTS. This spec-

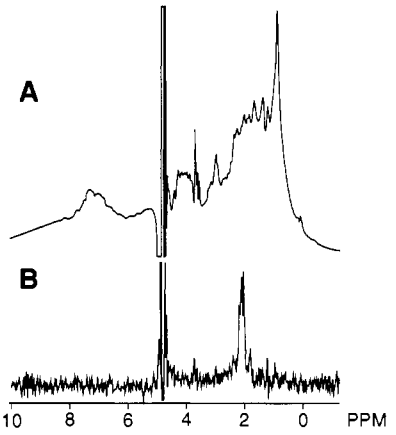

FIGURE 4: Proton (A) and echo difference (B) spectra of [*methyl*-^{13}C]methionine-labeled ΔMTS. A total of 4096 scans were obtained in blocks of 64 scans with on- and off-resonance ^{13}C irradiation. Δ, the time required for the resonances of the ^{13}C-labeled methyl protons to dephase by 180°, was set at 3.4 ms. The echo difference spectrum was obtained with broad-band ^{13}C decoupling. Spectra were obtained at 500 MHz with 8K data points, a sweep width of 8 kHz, a 90° ^{1}H pulse, and a repetition rate of 1.5 s at 25 °C. The sample contained 1.2 mM ΔMTS in 5 mM [$^{2}H_{11}$]Tris, pH 7.6, containing 50 mM NaCl, 0.1 mM DTT, and 0.1 mM EDTA.

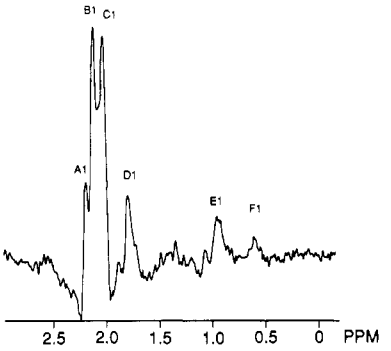

FIGURE 5: Echo difference spectrum of the methionine methyl proton region of [*methyl*-^{13}C]methionine-labeled ΔMTS obtained with broad-band ^{13}C decoupling. Experimental conditions were the same as those described in Figure 4.

trum is characteristic of a protein with a molecular mass of 66 kDa. Most resonances both upfield and downfield from the residual HDO signal appear as a complex envelope of lines. The chemical shift for the methyl protons of methionine are expected at approximately 3.1 ppm (Wuthrich, 1986). Proton spin-echo difference spectra with on- and off-resonance ^{13}C decoupling were used to selectively extract protons directly bonded to the ^{13}C-enriched methyl carbons (Figure 4B). The methyl proton resonances appear as singlets since broad-band carbon-13 decoupling was applied during acquisition (Figure 4B). Six resonances, labeled A1 through F1, are selectively extracted in the echo difference spectrum (Figure 5). Chemial shifts of these resonances range from 2.18 to 0.59 ppm (Figure 5). Resonances A1 through D1, with chemical shifts between 2.18 and 1.79 ppm, are from the methyl protons of the 17 methionine residues. However, the resonance at 0.95 ppm, E1, most likely results from detection at natural abundance level of the methyl resonances (Leu, Ile, and Ala) comprising the large peak at 0.95 ppm in the ^{1}H NMR spectrum of ΔMTS (Figure 4A). The small resonance labeled F1 is of unkown origin but was consistently reproducible. attempts at correlating the ^{1}H and ^{13}C chemical shifts with the 2D forbidden echo technique of Bax et al. (1983) were only partially successful due to the rapid longitudinal and transverse relaxation rates of the methyl resonances in a protein

Table III: Dissociation Constants of Methionyl-tRNA Synthetase–Ligand Complexes[a]

complex	K_D (mM) from Δδ of resonance B	M	n^b
E–L-methionine	0.11 ± 0.09[d]	*c*	1.0
E–L-homocysteine	11 ± 2	*c*	1.0
E–L-norleucine	7 ± 4	*c*	1.0
E–L-ethionine	3.3 ± 0.5	*c*	1.0
E–ATP		1.1 ± 0.2	0.9

[a] Determined by least-squares analysis of the titration data. Components present were 10 mM [$^{2}H_{11}$]Tris, 50 mM NaCl, 0.1 mM DTT, and 0.1 mM EDTA at pH 7.6. T = 25 °C. [b] Number of identical noninteracting sites on the synthetase. [c] Chemical shift of resonance did not change as a function of substrate concentration. [d] Average value from two separate titration experiments.

of this size. However, the spectra (Figures 4 and 5) clearly demonstrate the ability to selectively extract and observe proton resonances from a large protein with these techniques. Due to the much poorer resolution of individual methionine methyl resonances in the ^{1}H dimension, titrations with cognate and noncognate ligands were performed by monitoring the ^{13}C chemical shifts of the enriched methyl carbons directly by ^{13}C NMR with broad-band proton decoupling.

Effect of L-*Methionine, Adenosine, and MgPP on the* ^{13}*C Chemical Shifts.* Titration of ΔMTS with the cognate amino acid, L-methionine, resulted in a progressive upfield chemical shift of resonance B (Figure 6A,B) which could be fit by a calculated titration curve with a dissociation constant of 0.11 ± 0.09 mM and 1.0 binding site per ΔMTS monomer (Table III). The dissociation constant so obtained is in the range of the K_D value (0.08 ± 0.02 mM; Blanquet et al., 1975) obtained by equilibrium dialysis, suggesting active-site binding for L-methionine under the conditions of the NMR experiment. Chemical shift changes were also observed in the region of resonances D through L upon addition of L-methionine (Figure 6B). However resonance overlap in this part of the spectral region prohibited monitoring chemical shift changes as a function of the concentration of added L-methionine. Resolution of two new resonances were observed upfield of resonance L, L1 and L2, upon saturation of the enzyme with L-methionine (Figure 6B). Resonance L2 was observed to progressively shift upfield as a function of added cognate amino acid. Although the original position of this peak is unknown, it must correspond to at least a 0.3 ppm upfield shift. Resonances A, C, and M all failed to show any significant change in chemical shift as a consequence of saturating the enzyme with cognate amino acid (Figure 6A,B).

The addition of 3.3 mM adenosine to 0.50 mM ΔMTS in the presence of 5.0 mM L-methionine gave the ^{13}C NMR spectrum shown in Figure 6C. From the concentrations used, and the dissociation constant of adenosine from the ΔMTS–Met complex, K_D = 0.76 mM (Blanquet et al., 1975), the synthetase was estimated to be 80% saturated with adenosine under the conditions of the NMR experiment. Resonance M was shifted 0.12 ppm downfield upon addition of saturating adenosine (Table IV). Comparison of the ternary ΔMTS–Met–adenosine complex (Figure 6C) with the binary ΔMTS–Met complex (Figure 6B) also shows that ternary comlex formation produced minor changes in both the chemical shift and intensity of resonances in the region from 16.7 to 14.5 ppm. Again, due to the spectral overlap in this region, individual chemical shift changes were difficult to interpret. The addition of 6 mM MgPP to the ternary ΔMTS–Met–adenosine complex resulted in resonance M being shifted downfield an additional 0.12 ppm (Table IV). Additional

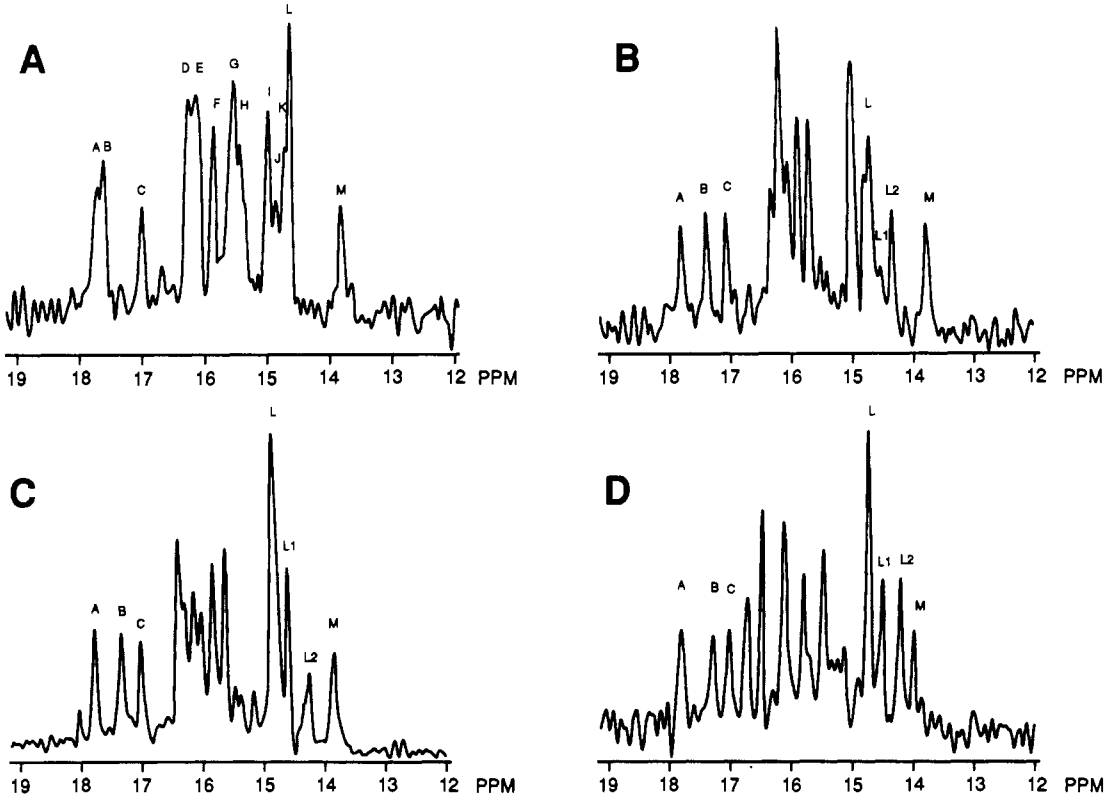

FIGURE 6: Effect of ligands on the ^{13}C NMR spectrum of [*methyl*-^{13}C]methionine-labeled ΔMTS. (A) The proton-decoupled ^{13}C NMR spectrum of 0.50 mM [*methyl*-^{13}C]methionine-labeled ΔMTS. Effects on the ^{13}C-enriched methyl resonances of ΔMTS upon the addition of 5.0 mM L-methionine to ΔMTS (B), the addition of 3.3 mM adenosine to the binary ΔMTS–Met complex (C), and the addition of 6 mM MgPP to the ternary ΔMTS–Met–adenosine complex (D). NMR spectra were obtained under the conditions described in Figure 3.

movement of resonances in the region between 16.7 and 14.5 ppm (Figure 6D) were also observed upon formation of the ΔMTS–Met–adenosine–MgPP complex.

Effect of Cognate and Noncognate Amino Acids on the ^{13}C Chemical Shifts. Changes in the ^{13}C NMR spectrum of labeled ΔMTS were monitored as a function of cognate and noncognate amino acids. Due to the complexity of the ^{13}C NMR spectrum in the region between 16.5 and 14.5 ppm, only the chemical shifts of resonances A, B, C, and M could be accurately monitored as a function of ligand concentration (Figure 6A). Titration of ΔMTS with L-methionine, L-homocysteine, L-ethionine, or L-norleucine all produced progressive upfield chemical shifts in resonance B (Table IV). No changes in the chemical shifts of resonance A, C, or M were observed in the presence of saturating concentrations of any of these ligands. The dissociation constant for each of the ligands was determined from titration curves monitoring the progressive upfield chemical shift of resonance B with increasing ligand concentration (Table III). The dissociation constants so obtained (Table III) were all in reasonable agreement with the values obtained by kinetic analysis on *E. coli* ΔMTS (Table II) or on *Bacillus stearothermophilus* MTS (Fersht & Dingwall, 1979). L-Methionine, the cognate amino acid, was found to produce the largest upfield shift in resonance B, 0.34 ppm. In contrast, saturating concentrations of L-homocysteine produced only a 0.17 ppm upfield shift in resonance B (Table IV). Changes in the chemical shift of resonance B as a consequence of active site binding of various amino acid ligands are summarized in Table IV. The upfield shift in resonance B decreases with saturating concentrations of the amino acid ligands in the order L-methionine > L-homocysteine, L-ethionine > L-norleucine. D-Methionine, L-homocysteinyl lactone, and L-*S*-ethylcysteine all failed to produce significant chemical shift changes in resonance B (Table IV), or in any of the other resolved resonances. D-Methionine and L-*S*-ethylcysteine are known not to support ATP–pyrophosphate exchange (Old & Jones, 1977), and no inhibition in the ATP–pyrophosphate exchange assay was observed with concentrations of L-homocysteinyl lactone up

Table IV: Changes in the Carbon-13 Chemical Shifts of the Methyl Resonances in [*methyl*-^{13}C]Methionine-Labeled Methionyl-tRNA Synthetase in Complexes with Ligands[a]

ligands	amino acid R group	$\Delta\delta_{max}$ (ppm) of resonance B	M
L-methionine	$-CH_2CH_2SCH_3$	0.34	*b*
D-methionine		*d*	*d*
L-homocysteine	$-CH_2CH_2SH$	0.17	*b*
L-homocysteinyl lactone		*c*	*c*
L-ethionine	$-CH_2CH_2SCH_2CH_3$	0.18	*b*
L-norleucine	$-CH_2CH_2CH_2CH_3$	0.09	*b*
L-*S*-ethylcysteine	$-CH_2SCH_2CH_3$	*e*	*e*
ATP		0.10	−0.33
MgATP		0.10	−0.15
adenosine + L-methionine		0.30	−0.09
adenosine + L-methionine + MgPP		0.34	−0.24

[a] Determined from the difference in chemical shifts of the resonances in the absence and presence of saturating amounts of ligand. Errors are ±0.03 ppm. Upfield chemical shifts are reported as positive and downfield as negative. [b] Within error no change in chemical shift observed at concentrations of ligand sufficient to saturate MTS under conditions of the NMR experiment. [c] Within error no change in the chemical shift of the enriched methyl resonances observed when up to 8.8 mM L-homocysteinyl lactone was added to 0.54 mM ΔMTS. [d] Within error no change in the chemical shifts of the enriched methyl resonances observed when up to 6 mM D-methionine was added to 0.55 mM ΔMTS. [e] Within error no change in the chemical shifts of the enriched methyl resonances observed when up to 7.0 mM L-*S*-ethylcysteine was added to 0.55 mM ΔMTS.

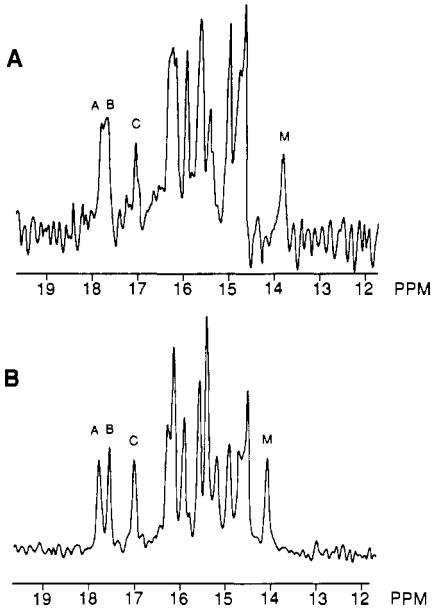

FIGURE 7: Proton-decoupled ^{13}C NMR spectra of (A) ΔMTS and (B) ΔMTS in the presence of 18.4 mM ATP. The sample contained 0.46 mM [*methyl*-^{13}C]methionine-labeled ΔMTS in 5 mM [$^{2}H_{11}$]Tris buffer, pH 7.6, containing 50 mM NaCl, 0.1 mM EDTA, and 0.1 mM DTT. Spectra were obtained under the conditions described in Figure 3.

to 8 mM. Therefore, effects on resonance B are confined to ligands that are known to bind MTS and catalyze either an ATP–pyrophosphate exchange or an ATP pyrophosphatase activity.

Effect of Nucleotides on the ^{13}C Chemical Shifts. Changes in the chemical shifts of the methionine methyl resonances upon addition of saturating concentrations of ATP are shown in Figure 7. Titration with ATP caused progressive chemical shift changes of resonances B and M. Resonance B was shifted upfield by 0.10 ppm, and resonance M was shifted downfield by 0.33 ppm (Figure 7; Table IV). No change in the chemical shift of resonances A and C was detected (Figure 7). Minor chemical shift changes in the region between 16.5 and 14.5 ppm were also observed in the presence of saturating concentrations of ATP. However, these changes were much smaller than those observed in the presence of saturating concentrations of amino acid analogues (Figure 6 and 7). The progressive downfield chemical shift of resonance M upon addition of ATP would be well fit (Figure 8) by a Scatchard plot using the parameters of Table III. The K_D value obtained from the progressive downfield chemical shift of resonance M (1.1 ± 0.2 mM) is in agreement with the K_D value (1.2 mM) obtained by fluorescence experiments on the trypsin-modified enzyme (Blanquet et al., 1974). Titration of the ΔMTS–ATP complex with $MgCl_2$ resulted in a progressive upfield shift (0.18 ppm) of resonance M such that the total downfield shift of resonance M in the ΔMTS–ATP–Mg complex was only 0.15 ppm (Table III). A similar downfield shift of resonance M (0.12 ppm) was observed when adenosine was added to the ΔMTS–Met complex (Table III; Figure 6C).

Discussion

By use of a strain carrying the plasmid which overproduces ΔMTS, we were able to obtain approximately 3 mg of >95% pure ΔMTS per gram of cells (Table I; Figure 1). The purified synthetase exhibited an electrophoretic mobility on SDS–polyacrylamide gel electrophoresis consistent with it having a molecular mass of 66 000 daltons as predicted from the amino acid composition (Barker et al., 1982). The isolation procedure is rapid, less than 5 days, and has an overall recovery of 64%. Both of these criteria, high yield per gram of cells and ease of purification, are necessary before stable isotopes can be efficiently incorporated for physical studies of wild-type and mutant proteins.

Table II summarizes some of the catalytic and physical properties of ΔMTS purified from the overproducing strain. The Michaelis constants for ATP and L-methionine in the ATP–pyrophosphate exchange reaction were found to agree with those previously obtained for both the native and trypsin-modified enzymes (Table II). Dissociation constants determined from monitoring the progressive chemical shift changes in resonances B and M upon addition of ligands (Table III) were also found to be consistent with those previously published for both the trypsin and native methionyl-tRNA synthetases. The native molecular mass of ΔMTS (66 kDa, Figure 2) was consistant with it being a monomer under the conditions of the NMR experiment. This was expected since ΔMTS contains only 17 additional amino acids at the carboxy terminus compared to the trypsin-modified enzyme.

Incorporation of [*methyl*-^{13}C]methionine into ΔMTS was accomplished by growing an *E. coli* strain auxotrophic for methionine and carrying the plasmid which overproduces ΔMTS in the presence of [*methyl*-^{13}C]methionine. Of the 17 methionine residues in ΔMTS, 13 ^{13}C-enriched methyl resonances could be observed by ^{13}C NMR spectroscopy (Figure 3). By comparison, only 4 of the methyl ^{1}H resonances could be selectively observed with proton-detected heteronuclear edited difference spectra due to the magnetic equivalence of the methyl protons (Figures 4 and 5). Hence, ligand-promoted conformational changes in the synthetase could be best monitored by direct ^{13}C NMR spectroscopy.

As shown in Figure 6, ligand binding induces a number of changes in the chemical shifts of the ^{13}C-enriched methyl resonances. Unfortunately, it is not yet possible to assign any of these resonances to specific methionines in the primary sequence of ΔMTS, although assignments can be made by site-directed mutagenesis. Chemial shift changes in the ^{13}C-enriched methyl resonances upon ligand binding could result from changes in the electronic environment of the resonance caused directly by the bound ligand or as the result of a ligand-induced conformation change in the protein. Additionally, a ligand could exert both a direct and an indirect effect, ligand-induced conformational change, on a resonance. The delocalized nature of the chemical shift changes induced by L-methionine binding at the aminoacyl adenylate site (Figure 6A,B) are difficult to explain solely on the basis of direct effects. The binding of $tRNA^{fMet}$ to the native dimeric MTS has previously been shown to result in delocalized conformational changes in the synthetase (Ferguson & Yang, 1986).

Chemical shift changes occurring in the region 14.5–16.5 ppm were difficult to interpret due to resonance overlap. However, resonances A, B, C, and M could be closely followed during titration of ΔMTS with various amino acid ligands. No changes in resonances A, C, and M were observed upon addition of saturating concentrations of L-methionine or any of the amino acid ligands (Table IV). Resonance B exhibited a progressive upfield chemical shift upon addition of increasing concentrations of amino acid ligands which yielded dissociation constants consistent with active site binding and one binding site per ΔMTS monomer (Table III). However, the maximum chemical shift change of resonance B in the presence and absence of ligand varied depending on the nature of the amino acid ligand (Table IV). The cognate amino acid, L-methionine, was found to produce the largest chemical shift change, 0.34

ppm (Table IV). Resonance B exhibited only a 0.17 ppm chemical shift change upon addition of a saturating concentration of L-homocysteine. L-Homocysteine is presumably the target of the editing reaction of the methionyl-tRNA synthetase (Fersht & Dingwall, 1979). Saturating concentrations of L-ethionine, an unnatural amino acid which is larger than methionine, produced a 0.18 ppm shift in resonance B (Table IV). L-Ethionine has been shown to escape the editing reaction of MTS and to be transferred to tRNA (Fersht & Dingwall, 1979). Finally, L-norleucine, which is nominally isosteric with the cognate amino acid L-methionine, was found to produce only a 0.09 ppm chemical shift change in resonance B. L-Norleucine is interesting since it is both transferred to tRNA and presumably edited by MTS (Fersht & Dingwall, 1979). The C–C bond length, 1.54 Å, in norleucine is only 0.27 Å shorter than the C–S bond length, 1.81 Å, in methionine, and the C–S–C and C–C–C bond angles differ only slightly, 105° and 108° for methionine and norleucine, respectively. However, these differences in bond length and bond angle are sufficient to induce conformational differences in the synthetase detectable by observation of resonance B. These differences do not represent a purely local effect on the structure of ΔMTS since differences in the chemical shifts of the ^{13}C-enriched methyl resonances in the region 14.5–16.5 ppm are also observed. Thus, depending on the exact nature of the amino acid side chain, the synthetase can exhibit different conformations that appear not to be localized to the amioacyl adenylate site but to be propagated throughout the protein. These conformational changes require binding of the amino acid to the active site since ligands known not to bind at the aminoacyl adenylate site (D-methionine, L-*S*-ethylcysteine, and L-homocysteinyl lactone) do not produce observable changes in the chemical shifts of the enriched methyl resonances. This represents the first evidence that the methionyl-tRNA synthetase can exist in different conformational states depending on the exact nature of the amino acid ligand bound at the aminoacyl adenylate site. Since it is well-known that only L-amino acids bind to MTS, it appears that distances from the primary amino group to both the sulfur and the methyl group are critical for inducing the proper conformational change. This conformational change is not necessary for activation of the amino acid since both cognate and noncognate amino acids can be activated, but may be involved in discrimination by the enzyme between cognate and noncognate amino acids.

Interestingly, saturating concentrations of ATP also produce a small, 0.10 ppm, upfield chemical shift in resonance B. Since both L-amino acids and ATP produce upfield shifts in resonance B, resonance B could be positioned near both the carboxylate of the amino acid and the phosphate groups of ATP. A negative charge on the amino acid is not necessary to induce the upfield chemical shift in resonance B since the addition of L-methioninol, an amino acid analogue lacking the carboxylate group, was found to produce a 0.40 ppm upfield shift (data not shown).

The ligand-dependent conformational changes detected by NMR suggest that modeling studies, based on the crysallographic structure of trypsin MTS (Brunie et al., 1987)), may not permit details of the structural mechanisms utilized in distinguishing between cognate and noncognate complexes to be fully elucidated. These studies also suggest that caution should be taken when site-directed mutagenesis studies are used to probe the role of individual amino acid site chains in catalysis, since the effect of the mutation on the conformational states available to MTS is unknown in the absence of direct

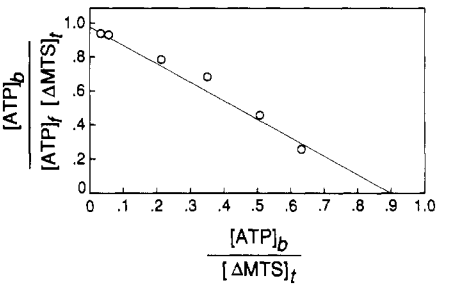

FIGURE 8: Scatchard plot describing the binding of ATP to ΔMTS by measuring the chemical shift change of resonance M upon addition of ATP. Conditions are described in Figure 7. The data were fit by linear regression with a K_D = 1.1 ± 0.2 (Table III).

Table V: Correlation between the Negative Charges Introduced by Ligands at the Aminoacyl Adenylate Site of the Truncated Methionyl-tRNA Synthetase and Extent of the Chemical Shift of Resonance M[a]

ligands	$\Delta\delta_{max}$ of resonance M[b]	negative charges	
		amino acid site	nucleotide site
ATP	−0.33		4 e^-
MgATP	−0.15		2 e^-
adenosine + L-Met	−0.09	1 e^-	
adenosine + L-Met + MgPP	−0.24	1 e^-	2 e^-

[a] The charges associated to phosphate groups are expressed as electrons and calculated from Fayat et al. (1977). Downfield chemical shifts are reported as negative. [b] The maximum difference in the chemical shift of resonance M determined in the presence and absence of saturating amounts of ligands. NMR spectra were obtained as described in Figure 3.

structural data on the various solution conformations.

The addition of ATP to ΔMTS resulted in a downfield chemical shift of resonance M (Figure 7; Table IV). The Scatchard plot monitoring the progressive downfield chemical shift of resonance M with increasing concentrations of ATP yielded a K_D (1.1 ± 0.2 mM) for the binding of ATP to ΔMTS in agreement with previously published values indicating active site binding of ATP under the conditions of the NMR experiment (Figure 8). Addition of Mg^{2+} to the ΔMTS–ATP binary complex resulted in resonance M being shifted upfield (0.18 ppm) toward its position in the absence of substrates (Table IV). Increasing the MgATP concentration 4-fold had no further effect on the chemical shift of resonance M. Comparison of the overall conformational changes, as measured by the chemical shifts of the ^{13}C-enriched methyl resonances, induced by methionine and ATP binding (Figures 6 and 7) suggests that ATP binding produces a much more localized conformational change in the synthetase than amino acid binding.

The binding of adenosine to the ΔMTS–methionine binary complex produced a 0.09 ppm chemical shift change in resonance M that increased to 0.24 ppm upon the addition of MgPP (Table IV). The downfield chemical shift of resonance M could be correlated with the negative charge introduced by the ligands at the aminoacyl adenylate site a shown in Table V. The negative charges, expressed as electrons, present at the aminoacyl adenylate site were calculated from the data of Fayat et al. (1977). Resonance M is shown to be shifted downfield by approximately 0.08 ppm for every negative charge introduced by a ligand at the amioacyl adenylate site (Table V). The presence of adenosine or ATP is necessary to observe this effect since the binding of amino acids alone does not alter the chemical shift of resonance M (Table IV). Couplings between ligands, i.e., changes in the dissociation constant of one ligand upon binding of another ligand at the

aminoacyl adenylate site, have previously been shown to be dependent on the negative charge introduced by the ligand (Fayat et al., 1977). However, these couplings were not correlated with conformational changes in the synthetase induced by substrate binding. It has recently been suggested that catalysis by some aminoacyl-tRNA synthetases results solely from the use of enzyme–substrate binding energy (Borgford et al., 1987; Fersht, 1987). The correlation of the number of negative charges introduced by the ligands, at the aminoacyl adenylate site, with the chemical shift of resonance M may be monitoring discrete steps of enzyme–substrate complementarity along the reaction path toward products.

A short region of homology, viewed as the signature sequence, near the N-terminus of the primary sequence has been observed in a number of aminoacyl-tRNA synthetases (Webster et al., 1984). This region has recently been suggested to be involved in forming part of the binding site for ATP during aminoacyl adenylate synthesis in those enzymes having this sequence (Borgford et al., 1987). This region in MTS, His_{21}-Leu_{22}-Gly_{23}-His_{24}-Met_{25}, has been predicted to be in a loop connnecting an α-helix to a β-sheet (Strazyk et al., 1987). The corresponding region in the tyrosyl-tRNA synthetase has been shown to be located in a loop, and it has been suggested that this region of the protein is highly mobile and wraps itself around the substrates in an induced-fit mechanism (Fersht, 1987). It is interesting to speculate that resonance M corresponds to Met-25 and that changes in the chemical shift observed in resonance M, as a result of the negative charge introduced at the aminoacyl adenylate site, result from either an "edge" ring current effect from the adenine ring or from alteration of the electron density on the sulfur. This Met residue is located next to His-24 which, based on homology with the tyrosyl-tRNA synthetase, is predicted to form part of the binding site for ATP in the ΔMTS–Met–AMP–PP_i complex (Borgford et al., 1987; Fersht, 1987). The protonation state of His-24 may be dependent on the number and location of negative charges at the active site and thus may indirectly affect the electronic environment around the sulfur of Met-25. Methionine residues 333 and 535 have also been shown to be located near the ATP binding site (S. Brunie, personal communication), and it is interesting to further speculate that resonance B, which is presumed to be located near the aminoacyl adenylate site, could in fact be one of these methionine residues.

These studies clearly demonstrate that stable isotopic enrichment can be used to detect conformational changes in a large protein by ^{13}C NMR spectroscopy and that the conformation of MTS is sensitive to both the nature of the amino acid side chain and the negative charge at the aminoacyl adenylate site. The wide range of chemical shift changes produced by the binding of amino acid ligands suggests that substrate-induced conformational changes in ΔMTS are delocalized and propagated throughout the entire protein. In contrast, the binding of nucleotides produces much more localized conformational changes in ΔMTS. We are currently in the process of assigning methionine residues 25, 333, and 535 using site-directed mutagenesis. Histidine residues within the signature sequence are also being probed by site-directed mutagenesis and incorporation of [2-^{13}C]His into the methionyl-tRNA synthetase.

ACKNOWLEDGMENTS

I thank Dr. David G. Barker for his generous gift of the plasmid MTS-a5 carrying the gene encoding the truncated methionyl-tRNA synthetase, Dr. Simone Brunie for helpful discussions, and J. Scott Williams for determining the kinetic constants for ATP and methionine. I thank John Coale for his technical assistance during the course of this work.

REFERENCES

Barker, D. G., Ebel, J.-P., Jakes, R., & Bruton, C. J. (1982) *Eur. J. Biochem. 127*, 449.

Bax, A., Griffey, R. H., & Hawkins, B. L. (1983) *J. Magn. Reson. 55*, 301.

Blakely, R. L., Cocco, L., London, R. E., Walker, T. E., & Matwiyoff, N. A. (1978) *Biochemistry 17*, 2284.

Blanquet, S., Iwatsubo, M., & Waller, J.-P. (1973) *Eur. J. Biochem. 36*, 213.

Blanquet, S., Fayat, G., & Waller, J.-P. (1974) *Eur. J. Biochem. 44*, 343.

Blanquet, S., Fayat, G., & Waller, J.-P. (1975) *J. Mol. Biol. 94*, 1.

Borgford, T. J., Gray, T. E., Brand, N. J., & Fersht, A. R. (1987) *Biochemistry 26*, 7246.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248.

Brunie, S., Mellot, P., Zelwer, C., Risler, J.-L., Blanquet, S., & Fayat, G. (1987) *J. Mol. Graphics 5*, 18.

Cassio, D., & Waller, J.-P. (1974) *Eur. J. Biochem. 20*, 283.

Eakin, R. T., Morgan, L. O., & Matwiyoff, N. A. (1975) *Biochem. J. 152*, 529.

Fayat, G., & Waller, J.-P. (1974) *Eur. J. Biochem. 44*, 335.

Fayat, G., Fromant, M., & Blanquet, S. (1977) *Biochemistry 16*, 2570.

Ferguson, B. Q., & Yang, D. C. H. (1986) *Biochemistry 25*, 2743.

Fersht, A. R. (1987) *Biochemistry 26*, 8031.

Fersht, A. R., & Dingwall, C. (1979) *Biochemistry 18*, 1238.

Griffey, R. H., Redfield, A. G., Loomis, R. E., & Dalquist, F. W. (1985) *Biochemistry 24*, 817.

Heinrikson, R. L., & Hartley, B. S. (1976) *Biochem. J. 105*, 17.

Hines, D., & Chlebowski, J. F. (1987) *Biochem. Biophys. Res. Commun. 144*, 375.

Hyafil, F., Jacques, Y, Fayat, G., Fromant, M., Dessen, P., & Blanquet, S. (1976) *Biochemistry 15*, 3678.

Jones, W. C., Jr., Rothgeb, T. M., & Gurd, F. R. N. (1976) *J. Biol. Chem. 251*, 7452.

Mulvey, R. S., & Fersht, A. R. (1976) *Biochemistry 15*, 243.

Old, J. M., & Jones, D. S. (1977) *Biochem. J. 165*, 367.

Rigby, P. W. J., Gething, M. J., & Hartley, B. S. (1976) *J. Bacteriol. 125*, 728.

Schimmel, P. R., & Soll, D. (1979) *Annu. Rev. Biochem. 48*, 601.

Shaka, A. J., Keeler, J., & Freeman, R. (1983) *J. Magn. Reson. 53*, 313.

Starzyk, R. M., Webster, T. A., & Schimmel, P. (1987) *Science (Washington, D.C.) 237*, 614.

Webster, T. A., Tsai, H., Kula, M., Machie, G., & Schimmel, P. (1984) *Science (Washington, D.C.) 226*, 1315.

Wuthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, Wiley, New York.