Reactions of Thio Analogues of Adenosine 5′-Triphosphate Catalyzed by Methionyl-tRNA Synthetase from *Escherichia coli* and Metal Dependence of Stereospecificity†

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ABSTRACT: Two aspects of the reactions of the diastereomers of adenosine 5′-*O*-(1-thiotriphosphate) (ATPαS) and adenosine 5′-*O*-(2-thiotriphosphate) (ATPβS) catalyzed by methionyl-tRNA synthetase (Met-tRNA synthetase) were investigated. First, the kinetic parameters of the thio analogues relative to adenosine 5′-triphosphate (ATP) were compared with Mg(II) as the activating ion in the overall aminoacylation reaction, in the pyrophosphate exchange reaction, and in the interchange reaction in which the β- and γ-phosphates of ATP or its thio analogues are interchanged in the absence of pyrophosphate. In aminoacylation the V_{max} values for the γS and the preferred diastereomers of the αS and βS Mg(II)–thionucleotide complexes were reduced by factors ranging from 1.7 to 180 compared to the V_{max} value for ATP. In the exchange reaction, the thionucleotide rates with ATPγS and the preferred diastereomers of ATPαS and ATPβS were reduced by factors ranging from about 3 to 30 compared to rates with ATP. Aminoacylation rates at 1 mM nucleotide show that although exchange rates with thionucleotides are much lower than those with ATP as substrate, PP_i–thio-ATP exchange is faster than aminoacylation with ATPβS and ATPγS, as for ATP itself. Aminoacylation rates for ATPβS and ATPγS are lower than for ATP although in no case is the first step (exchange) rate determining, implying that the thiopyrophosphate moiety may affect the rate of the second step (aminoacyl-tRNA formation and product release). With ATPαS, aminoacylation is almost 3-fold faster than exchange. The mechanistic implications of the relative rates of the first step (exchange) and the overall reaction (aminoacylation) are discussed. The kinetic parameters for β-,γ-phosphate interchange of ATP catalyzed by Met-tRNA synthetase in the absence of added PP_i were investigated with [γ-^{32}P]ATP and ATPβS. Unlike the valyl-tRNA synthetase (Val-tRNA synthetase) where opposite diastereomers are preferred in aminoacylation and interchange, for the methionyl enzyme, the A isomer of ATPβS was preferred for all reactions. While the exchange and aminoacylation rates with ATPβSA were greatly reduced compared to rates with ATP, the V_{max} values for ATP and ATPβSA in the interchange reaction were approximately equal. Also, the interchange rate in the absence of PP_i with ATPβSA as substrate is only 40% lower than its exchange rate with 1 mM PP_i. These results led to the conclusion that interchange occurs while the nucleotide is bound to the enzyme. The second aspect investigated was the stereospecificity of the diastereomers in the Mg(II)-activated reaction and the reversal, if any, with Zn(II) as activating ion. In aminoacylation the MgATPαSA and MgATPβSA complexes were preferred over their B diastereomers by factors of more than 20 000 and 1000, respectively, the reaction of the B isomers being below the limits of detectability. With Zn(II) as activating divalent cation in the aminoacylation reaction, no activity was detected with ATPαSA or ATPαSB as substrate. Because of the inactivity of ATPαSB with Mg(II) or Zn(II) in the aminoacylation reaction, reversal of stereospecificity cannot be demonstrated directly, but absence of reversal is implied since one diastereomer, ATPαSA, is an active substrate for exchange with both metal ions and the other diastereomer, ATPαSB, is inactive with both. With ATPβS, the stereospecificity was reversed with zinc relative to MgATPβS. The ratio of the V_{max} values of the B isomer to that of the A isomer is 20 for ZnATPβS. In exchange, MgATPαSA and MgATPβSA were the preferred isomers by factors of 20 000 and 230, respectively. Also, the stereospecificity of the exchange reaction was reversed with ZnATPβS, the B isomer being favored by a factor of 5. With ATPαS reversal of the stereospecificity of the exchange reaction is unlikely, since activity with ZnATPαSB as substrate was undetectable. These results are consistent with the structure of the active metal–nucleotide complex in the Met-tRNA synthetase reaction pathway as a βγ chelate of ATP.

Aminoacyl-tRNA synthetases generally catalyze the production of aminoacyl-tRNA by a two-step mechanism: the activation of amino acid to aminoacyl adenylate with the concomitant release of PP_i,[1] followed by the transfer of the aminoacyl group to tRNA (Kisselev & Favorova, 1974). The first step is usually much faster than the second step and may be studied in most cases independently of aminoacylation by an ATP–PP_i exchange reaction in the absence of tRNA. In addition, a third reaction may be observed—the amino acid dependent interchange reaction in which the β-P and γ-P of ATP are interchanged in the absence of added PP_i. As demonstrated in previous reports (Rossomando et al., 1979; Smith & Cohn, 1981) the observed interchange reaction has contributions from both the exchange reaction, in which PP_i (SPP_i when ATPβS is the substrate) is released from the enzyme prior to interchange, and the direct interchange reaction, in which the reaction occurs on the enzyme surface and PP_i is not released by the enzyme. The relative contributions of the exchange-mediated interchange and the direct interchange to the observed reaction vary when noncognate amino acids or

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[1] Abbreviations: ATPαS, adenosine 5′-*O*-(1-thiotriphosphate); ATPβS, adenosine 5′-*O*-(2-thiotriphosphate); ATPγS, adenosine 5′-*O*-(3-thiotriphosphate); AMPS, adenosine 5′-*O*-thiomonophosphate; BSA, bovine serum albumin; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; PEI–cellulose, poly(ethylenimine)–cellulose; PP_i, inorganic pyrophosphate; SPP_i, inorganic thiopyrophosphate; TEA, triethylamine; DEAE, diethylaminoethyl.

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thio analogues of ATP are substituted for the normal substrates.

Thio analogues of ATP in which a nonbridge oxygen is replaced by sulfur on the α-, β-, or γ-P of ATP have been used to investigate the reaction pathways of phenylalanyl-tRNA synthetase (Phe-tRNA synthetase) (Pimmer et al., 1976) and valyl-tRNA synthetase (Val-tRNA synthetase) (Rossomando et al., 1979) from *Escherichia coli* and Phe-tRNA synthetase from yeast (von der Haar et al., 1977; Connolly et al., 1980). A pair of diastereomers, A and B, exist for both ATPαS and ATPβS, and consequently, the stereospecificity with respect to the α- and β-P of the respective ATPS substrates of the aminoacyl-tRNA synthetase may be investigated. In addition, the ligand structure of the active metal–nucleotide complex in the synthetase reaction may be elucidated. It has been shown that Mg(II) is chelated to ATPS through the oxygen atom while Zn(II) is chelated through the sulfur atom (Jaffe & Cohn, 1978a). Therefore, at the β-P, for example, MgATPβSA has the same geometry as ZnATPβSB. If the stereospecificity of an enzyme is reversed from one ATPβS stereoisomer to the other when the activating metal is changed from Mg(II) to Zn(II), it may be concluded that the metal is coordinated to the nucleotide at the β-P at a rate-determining step in the reaction. Such a reversibility of stereospecificity has been demonstrated for a number of enzymes (Eckstein, 1979).

In this report, ATPαSA, ATPαSB, ATPβSA, ATPβSB, and ATPγS were tested as substrates with Mg(II) and Zn(II) to probe the pathway and divalent cation dependent stereospecificity of Met-tRNA synthetase from *E. coli*. ATPβSA and ATPβSB were compared to ATP as substrates for the β-P,γ-P interchange reaction. All five analogues were tested for activity in the aminoacylation reaction and in the exchange reaction with PP_i. The PP_i "exchange" reaction with the thio analogues as substrates may be formulated as follows:

$$\text{ATPS } (\beta \text{ or } \gamma) + \text{AA} \rightleftarrows \text{AMP-AA} + \text{SPP}_i \quad (1)$$

$$\text{AMP-AA} + \text{PP}_i \rightleftarrows \text{ATP} + \text{AA} \quad (2)$$

so that total nucleotide is conserved but there is a conversion of ATPS to ATP. Since the rate of PP_i exchange with ATP as substrate (step 2) is very fast, any decrease in the exchange rate with ATPβS or ATPγS relative to ATP can be ascribed to a decrease in the rate of the first reaction, aminoacyl adenylate formation from ATPβS or ATPγS, because the second reaction, i.e., ATP formation, is identical with that with ATP as substrate.

Materials and Methods

ATPγS, ADPβS, and AMPS were purchased from Boehringer-Mannheim. For removal of contaminating ADP, ATPγS was chromatographed on a DEAE-Sephadex A-25 column (21 × 1.6 cm) with a TEA/CO_3 gradient (0.2–0.6 M), pH 7.6. ATP and inorganic pyrophosphatase were obtained from Sigma, hexokinase was from Worthington, $tRNA_f^{Met}$ was from Plenum, and spermidine was from Calbiochem. [^{32}P]PP_i (19.3 Ci/mmol) was obtained from New England Nuclear and [γ-^{32}P]ATP (17.9 Ci/mmol) was from Amersham. [^{14}C]Met (50 mCi/mmol) was obtained from Schwarz/Mann. The acid forms of Hepes and EDTA were purchased from Sigma and Fisher, respectively. Magnesium and zinc acetate were from Baker. All other chemicals were reagent grade or purest preparation available. Amino acids, Hepes, spermidine, and PP_i were treated with Chelex-100 (Bio-Rad) to remove contaminating metal ions. BSA was dialyzed extensively against 10 mM Hepes and Chelex-100. Contaminating metal ions were removed from ATP and its thio analogues by extraction with 1% 8-hydroxyquinoline in chloroform. Instabray scintillation solution was from Yorktown Research and PEI–cellulose plates were purchased from E. Merck. Arginine kinase was prepared from lobster muscle as described previously (Buttlaire & Cohn, 1974).

ATPαSB, ATPβSA, and ATPβSB were prepared as previously reported (Eckstein & Goody, 1976; Jaffe & Cohn, 1979a). ATPαSA was prepared by the reaction of AMPS with ATP catalyzed by adenylate kinase, followed by reaction with pyruvate kinase (Jaffe & Cohn, 1978b) with the following modifications: the reaction mixture (1 mL) containing ATPαSA was treated with 25 μmol of EDTA and the protein removed by passage over a G-25 column (25 × 0.6 cm) in two 0.5-mL aliquots. The product was eluted with 50 mM TEA/CO_3, pH 7.6, and evaporated to dryness by rotary evaporation. For removal of contaminating ATP, the ATPαSA mixture was redissolved in 0.5 mL of 0.1 M potassium glycine, pH 8.6, 10 mM $MgCl_2$, 15 mM Arg, and 50 μg of arginine kinase. After 15 min, all of the ATP was degraded to ADP as judged by high-pressure liquid chromatography. The reaction mixture was treated with Chelex-100, and the contaminants (AMPS, ADPαSA, and ADP) were separated from ATPαSA by chromatography on a DEAE-Sephadex A-25 column (25 × 1.6 cm) with TEA/CO_3 gradient (0.2 to 0.6 M), pH 7.6. Met-tRNA synthetase and Val-tRNA synthetase were purified from *E. coli* K12 (Grain Processing Corp.) as described previously (Rossomando et al., 1979).

The aminoacylation reaction was carried out under the following conditions: 40 mM Hepes, pH 8.0, 6 mM 2-mercaptoethanol, 0.2 mg/mL BSA, 1 mM spermidine, 5 μM $tRNA_f^{Met}$, 100 μM [^{14}C]Met, and from 0.025 to 4.8 mM ATP or its thio analogue in a total volume of 50 μL. The concentration of magnesium acetate in the assay mixture was 2 mM in excess of the nucleotide concentration. The zinc acetate concentration was 1 mM in excess of the nucleotide concentration. Higher levels of magnesium or zinc were found to be inhibitory. Thus, these concentrations were used in all of the experiments to obtain maximum activity. Reactions were initiated by addition of enzyme, then proceeded for 10–20 min at 30 °C, and were stopped with 2 mL of 5% trichloroacetic acid. The amount of [^{14}C]Met-tRNA formed was determined by liquid scintillation counting as previously described (Smith & Cohn, 1981).

The interchange reaction was monitored under the following conditions: 40 mM Hepes, pH 8.0, 6 mM 2-mercaptoethanol, 0.2 mg/mL BSA, 2 mM Met, 0.1–3.0 mM [^{32}P]ATP or unlabeled thio analogue, and magnesium acetate 2 mM in excess of nucleotide or zinc acetate 1 mM in excess of nucleotide. The final volume was 100 μL and the rate of the reaction was determined at 30 °C. With ATP, the production of [β-^{32}P]ATP from [γ-^{32}P]ATP was followed at 10–12-min intervals in four 20-μL aliquots of the reaction mixture. Reactions were terminated by the addition of 2 μL of a 40 mM solution of glucose containing 4 μg of hexokinase; 1 μL of 0.25 M magnesium acetate was also added to those reaction mixtures not already containing magnesium. Each reaction mixture (10 μL) plus 2 μL of a 5 mM solution of unlabeled ADP were spotted on a PEI–cellulose plate. The products were separated by thin-layer chromatography and counted by liquid scintillation counting as previously described (Smith & Cohn, 1981). With thio analogues of ATP, reactions proceeded for 15–60 min and were monitored by high-pressure liquid chromatography on a Whatman Partisil 10 strong anion-ex-

Table I: Kinetic Parameters of the Aminoacylation Reaction of Met-tRNA Synthetase[a]

	Mg			Zn		
	K_m (mM)	V_{max} [nmol (min mg)$^{-1}$]	$V_{max,A}/V_{max,B}$	K_m (mM)	V_{max} [nmol (min mg)$^{-1}$]	$V_{max,A}/V_{max,B}$
ATP	0.092	5900		0.14	4900	
ATPγS	0.38	3400		0.26	740	
ATPβSA	0.54	630	>21 000	0.15	50	0.05
ATPβSB		not detectable		5.5	1000	
ATPαSA	0.60	32			not detectable	
ATPαSB		not detectable			not detectable	

[a] Rates were determined under the following conditions: 40 mM Hepes, pH 8, 6 mM 2-mercaptoethanol, 0.2 mg/mL BSA, 5 μM $tRNA_f^{Met}$, 1 mM spermidine, 100 μM [^{14}C]Met, and 0.025–4.8 mM nucleotide. The magnesium acetate concentration was 2 mM in excess of nucleotide concentration and the zinc acetate concentration was 1 mM in excess.

change column run at 2 mL/min with 0.6 M $NH_4H_2PO_4$ as solvent. Each assay mixture (20 μL) was injected and the extent of reaction was quantitated by integrating the area under the ATPβS and ATPγS peaks.

The exchange reaction with PP_i was monitored under the following conditions: 40 mM Hepes, pH 8.0, 6 mM 2-mercaptoethanol, 0.2 mg/mL BSA, 2 mM amino acid, 1 mM [^{32}P]PP_i, 1 mM ATP or thio analogue, and 4 mM magnesium acetate or 3 mM zinc acetate; final volume, 50 μL. Reactions were carried out at 30 °C for 15–180 min and were stopped with 1 μL (1 μg) of inorganic pyrophosphatase plus 1 μL of magnesium acetate (0.25 M) in those assay mixtures not already containing magnesium. A 10-μL aliquot of each reaction mixture to which 1 μL of unlabeled ATP (5 mM) was added was spotted on a PEI–cellulose plate. The products were separated by thin-layer chromatography, and the amount of ^{32}P incorporated into ATP was determined by liquid scintillation counting (Smith & Cohn, 1981).

Results

Aminoacylation. The kinetic parameters of the aminoacylation reaction catalyzed by Met-tRNA synthetase determined for MgATP and its thio analogues are given in Table I. Unlike Val-tRNA synthetase (Rossomando et al., 1979), no substrate inhibition was observed with MgATPβS as substrate for Met-tRNA synthetase. The maximum velocities decrease the closer the sulfur substitution is to the α-P of MgATP; relative to MgATP the V_{max} values for ATPγS, ATPβSA, and ATPαSA are reduced about 1.8-, 9-, and 180-fold, respectively (cf. Table I). In addition, the K_m values increase relative to MgATP by 4-fold with ATPγS to more than 6-fold with ATPαS, indicating weaker binding as the sulfur substitution approaches the site of ATPS cleavage. Met-tRNA synthetase is very specific with respect to the diastereomers of ATPαS and ATPβS. Reaction with MgATPβSB and MgATPαSB was undetectable [less than 0.03 nmol (min mg)$^{-1}$]. Inhibition by 1 mM ATPαSB could not be detected in the reaction with 1 mM ATPαSA as substrate.

The aminoacylation reaction was also investigated in the presence of Zn(II) as the activating metal ion as shown in Table I. The effect of Zn(II) is to reverse dramatically the stereospecificity of the enzyme from ATPβSA with Mg(II) to ATPβSB. For the βS analogue, the ratio $V_{max,A}/V_{max,B}$ is greater than 21 000 with Mg(II) and with Zn(II) equals 0.05. In addition, the V_{max} for ZnATPβSB is about 30% greater than that for ZnATPγS. Although the B isomer is preferred in the presence of Zn(II), the K_m for ZnATPβSA is more than 40-fold lower than that for ZnATPβSB. In fact, the K_m for ZnATPβSA is 3 times lower than that for MgATPβSA. Reactions with either isomer of ATPαS with Zn(II) were undetectable [less than 0.03 nmol (min mg)$^{-1}$]. Although the V_{max} for aminoacylation with MgATP is decreased by less than 20% when ZnATP is substituted as substrate, with ATPγS the rate is decreased more than 4-fold upon Mg(II) replacement with Zn(II) with the latter substrate.

Table II: Kinetic Parameters of the Interchange Reaction of Met-tRNA Synthetase[a]

	Mg		Zn	
	K_m (mM)	V_{max} [nmol (min mg)$^{-1}$]	K_m (mM)	V_{max} [nmol (min mg)$^{-1}$]
ATP	0.24	3700	0.16	2300
ATPβSA	3.6	4100	not detectable[b]	
ATPβSB	not detectable		not detectable[b]	

[a] Interchange rates were determined under the following conditions: 40 mM Hepes, pH 8, 6 mM 2-mercaptoethanol, 0.2 mg/mL BSA, 2 mM Met, and 0.10–3.0 mM [γ-^{32}P]ATP or unlabeled thio analogue of ATP. The magnesium acetate and zinc acetate concentrations were 2 and 1 mM, respectively, in excess of nucleotide concentration. [b] ATPβS breakdown was observed.

Interchange. The kinetic parameters of the interchange reaction for ATP and ATPβS were also determined (Table II). As with the aminoacylation reaction the V_{max} of interchange for ATP in the presence of Mg(II) is reduced slightly, by less than 40% when Zn(II) is substituted. Also similar to aminoacylation, ATPβSA in the presence of Mg(II) is the preferred isomer for interchange. The B isomer was neither a substrate nor an inhibitor of the interchange reaction with the A isomer as substrate. Unlike the kinetic parameters for aminoacylation, in the interchange reaction the V_{max} with Mg(II) as the activating ion for ATPβSA is about equal to the V_{max} for ATP. However, the K_m for ATPβSA in this reaction is more than 10-fold greater than that for ATP.

Interchange was not detected with either ATPβSA or ATPβSB in the presence of Zn(II). Attempts were made to observe the interchange reaction over a range from 0.3 to 3.0 mM ATPβS to rule out the possibility that interchange had not been detected due to either substrate inhibition or very weak binding.

Exchange. The exchange reaction of Met-tRNA synthetase was investigated with nucleotide and PP_i at 1 mM (Table III). The general pattern of rates is similar to the V_{max} values obtained for the aminoacylation reaction; the rates decrease as the sulfur approaches the α-P of ATP, and the A isomers of MgATPαS and MgATPβS were preferred. Unlike aminoacylation, in the exchange reaction with ATPαS, activity was observed for both Mg(II) and Zn(II) with the A isomer, and neither metal ion activated the reaction with the B isomer. Thus, there is no evidence for metal-dependent reversal of stereospecificity. Reactions with 1 mM ATPαSA in the presence of either Mg(II) or Zn(II) were not inhibited with 1 mM ATPαSB. As with aminoacylation, a reversal of stereospecificity for the ATPβS isomers was observed. The ratio

Table III: Exchange Rates of Met-tRNA Synthetase with 1 mM Nucleotide[a]

	Mg		Zn	
	v [nmol (min mg)$^{-1}$]	v_A/v_B	v [nmol (min mg)$^{-1}$]	v_A/v_B
ATP	50000		25000	
ATPγS	14000		950	
ATPβSA	1600	20000	190	0.19
ATPβSB	0.08		1000	
ATPαSA	7		0.6	
ATPαSB	not detectable		not detectable	

[a] Rates were determined under the following conditions: 40 mM Hepes, pH 8, 6 mM 2-mercaptoethanol, 0.2 mg/mL BSA, 2 mM Met, 1 mM [^{32}P]PP_i, 1 mM ATP or thio analogue, and either 4 mM magnesium acetate or 3 mM zinc acetate.

Table IV: Exchange Rates of Val-tRNA Synthetase with 1 mM Nucleotide[a]

	Mg		Zn	
	v [nmol (min mg)$^{-1}$]	v_A/v_B	v [nmol (min mg)$^{-1}$]	v_A/v_B
ATP	34000		3000	
ATPβSA	620	1240	23	0.34
ATPβSB	0.5		67	

[a] Rates were determined under the following conditions: 40 mM Hepes, pH 8, 6 mM 2-mercaptoethanol, 0.2 mg/mL BSA, 2 mM Val, 1 mM [^{32}P]PP_i, 1 mM nucleotide, and either 4 mM magnesium acetate or 3 mM zinc acetate.

of rates for ATPβSA:ATPβSB at 1 mM nucleotide was 20000 with Mg(II) and 0.19 with Zn(II).

The generality of the reversal of stereospecificity at the β-P of ATPβS was investigated with Val-tRNA synthetase. As shown in Table IV, at 1 mM nucleotide, the A isomer of ATPβS is preferred by more than 3 orders of magnitude in the presence of Mg(II), but the specificity is reversed in the presence of Zn(II). When these experiments were conducted at lower concentrations of ATPβS (to avoid possible anomalies caused by substrate inhibition), similar results were obtained.

Discussion

The structural and catalytic properties of aminoacyl-tRNA synthetases vary greatly (Kisselev & Favorova, 1974; Söll & Schimmel, 1974). Variability is also evident when the behavior with thio analogues of ATP as substrates is studied. In this report, it has been shown that the V_{max} values of aminoacylation decrease by 2 orders of magnitude as the sulfur substitution approaches the site of bond cleavage; i.e., the order of rates is ATPγS > ATPβS > ATPαS. Similar results were obtained for Val-tRNA synthetase from *E. coli* (Rossomando et al., 1979). However, with Phe-tRNA synthetase from *E. coli* the maximum velocities with all five isomers varied by no more than a factor of 3 (Pimmer et al., 1976), and with Phe-tRNA synthetase from yeast, the V_{max} for ATPαSA was greater than those for ATPβSA or ATPβSB (Connolly et al., 1980). The stereospecificity for the MgATPαS and MgATPβS isomers varies among these synthetases as well. Although ATPαSA is preferred in the aminoacylation reaction by both yeast Phe-tRNA synthetase (Connolly et al., 1980) and *E. coli* Met-tRNA synthetase, ATPβSB is the preferred isomer with yeast Phe-tRNA synthetase and ATPβSA is preferred by *E. coli* Met-tRNA synthetase. Furthermore, with *E. coli* Val-tRNA synthetase (Rossomando et al., 1979), unlike the methionyl enzyme, ATPβSB is preferred in the aminoacylation reaction, but ATPβSA is preferred in interchange for both enzymes. These differences may exemplify differences in the geometry of active sites of the various synthetases. Another kinetic difference is the substrate inhibition by ATPβSA and ATPβSB (greater with the B isomer) observed with Val-tRNA synthetase from *E. coli* (Rossomando et al., 1979), which was not observed in the *E. coli* Met-tRNA synthetase reaction.

The magnitude of the kinetic constants of the interchange reaction given in Table II demonstrates that the observed interchange reaction with Met-tRNA synthetase is distinct from the exchange reaction. The stereospecificity of the exchange and the aminoacylation reactions with MgATPβS was dramatically reversed with ZnATPβS, but in the interchange reaction neither diastereomer of ZnATPβS proved to be a substrate. Furthermore, with MgATPβSA the interchange rate in the absence of added PP_i is only 40% slower than the exchange rate at 1 mM PP_i. It has been shown that MgATP undergoes interchange predominantly by an exchange-mediated mechanism in the Met-tRNA synthetase reaction (Smith & Cohn, 1981); therefore, the ratio of rates for ATP:ATPβSA for the exchange and interchange reactions should be similar if interchange with MgATPβSA is also exchange mediated. At 1 mM nucleotide, the ratio of interchange rates for ATP:ATPβSA is about 3, but for exchange the ratio is over 30. Direct interchange, that is, interchange of the β- and γ-P of ATPβS without dissociation of SPP_i from the enzyme, contributes to the observed interchange reaction in other aminoacyl-tRNA synthetases as well. In the observed interchange reaction of Val-tRNA synthetase the contribution of direct interchange is large, while the contribution of the exchange-mediated reaction is negligible (Rossomando et al., 1979). Unlike the interchange reaction of either Met-tRNA synthetase or Val-tRNA synthetase, only 20% of the observed interchange of yeast Phe-tRNA synthetase was ascribed to the direct interchange reaction (Connolly et al., 1980).

Just as the exchange reaction may contribute to the observed interchange reaction, direct interchange may contribute to the observed PP_i exchange reaction. Since interchange is not detectable with Zn(II), this reaction cannot interfere in the determination of the ratio of exchange rates for ZnATPβSA:ZnATPβSB. With Mg(II), however, the ratio is artificially high because both direct interchange and exchange contribute to the apparent rate of exchange for the A isomer, but there is no contribution from interchange for the B isomer (cf. Table II). With MgATPβSA much of the apparent exchange rate is due to interchange; at 1 mM ATPβSA the measured rates of exchange and interchange are 1600 and 900 nmol (min mg)$^{-1}$, respectively. As a consequence of interchange, ATPβSA is converted to ATPγS, which in turn rapidly undergoes exchange [14000 nmol (min mg)$^{-1}$ at 1 mM ATPγS]. Nevertheless, a significant portion of the exchange rate is due to a true exchange since the apparent exchange rate is about 1.8-fold faster than interchange. As a result, the ratio of exchange rates for ATPβSA:ATPβSB may be distorted, but qualitatively it may be concluded that with Mg(II) the A isomer is preferred and with Zn(II) the specificity reverses.

The results given in Table V show that PP_i exchange rates with ATPβS as substrate are much lower than the rates with ATP. This observation demonstrates that the substitution of sulfur for oxygen on the phosphates of ATP affects the first step of aminoacylation. With ATPβS the second step of aminoacylation, transfer of methionyl adenylate to tRNA and release of Met-tRNA and AMP, is identical with that with ATP. Consequently, the rate of the second step with ATPβS as substrate should be identical with that with ATP, and the overall aminoacylation rate should be about equal to the slow

Table V: Exchange, Aminoacylation, and Interchange Rates of Met-tRNA Synthetase at 1 mM Nucleotide[a]

	exchange		aminoacylation		interchange	
	Mg	Zn	Mg	Zn	Mg	Zn
ATP	50000	25000	5900	4300	3000	2000
ATPγS	14000	950	2500	590	–[b]	–
ATPβSA	1600	190	410	43	900	–
ATPβSB	0.08	1000	–	150	–	–
ATPαSA	7	0.6	20	–	–	–
ATPαSB	–	–	–	–	–	–

[a] Rates were determined as previously described in Tables I–III except that the concentrations of nucleotide, Mg, and Zn were 1, 3, and 2 mM, respectively, for aminoacylation and interchange. For exchange, the magnesium acetate and zinc acetate concentrations were 4 and 3 mM, respectively. Rates are given in nmol (min mg)$^{-1}$]. [b] (–) indicates that the reaction was not detected.

exchange rate with ATPβSB. With MgATPγS as substrate, the exchange rate is reduced more than 3-fold compared to the rate with ATP, but it is still more than twice the aminoacylation rate with ATP as substrate; therefore, the aminoacylation rate with MgATPγS should be about the same as that with ATP. The results given in Table V show that the aminoacylation rates with MgATPγS and ATPβSA and ATPβSB are much lower than expected. Assuming that the K_{eq} of enzyme-bound aminoacyl adenylate formation with ATPγS or ATPβS is not greatly reduced (Lerman & Cohn, 1980) relative to that with ATP, it may be concluded that the thionucleotides influence both steps of aminoacylation. Perhaps the SPP_i remains bound to the enzyme after aminoacyl adenylate formation and so is able to decrease the rate of Met-tRNA formation or release.

Unlike the other substrates investigated, with ZnATPγS the rate of the exchange reaction is only 60% faster than aminoacylation, suggesting that the rate of the first step in aminoacylation contributes significantly to the overall rate. With MgATPαSA the aminoacylation rate is faster than the exchange rate, which would result if tRNA facilitated the release of SPP_i. This observation is qualitatively similar to the result with ATPβSB in the reactions catalyzed by Val-tRNA synthetase in which aminoacylation is more than 60-fold faster than exchange at 1 mM nucleotide (Rossomando et al., 1979).

As shown in Table V in both aminoacylation and exchange the stereospecificity for ATPβS in the presence of Mg(II) is reversed in the presence of Zn(II), indicating that the metal binds to the β-P of ATP during catalysis. With ATPαSA the exchange reaction was detected in the presence of either Mg(II) or Zn(II), but in aminoacylation the reaction was observed only with MgATPαSA, even though with Mg(II) the aminoacylation rate is greater than the exchange rate. With ZnATPαSA the aminoacylation rate could be 20-fold lower than the exchange rate before the limit of detection of the assay was reached. Although reversal of the specificity with ATPαSA was not observed, metal binding to the α-P cannot be entirely ruled out. The ZnATPαSB complex may bind to the enzyme in such a way that the sulfur is in a position so unfavorable for reaction that none is detected. The observations that both MgATPαSA and ZnATPαSA are substrates in the exchange reaction and that 1 mM ATPαSB is not an inhibitor of the reaction with 1 mM ATPαSA with either Mg(II) or Zn(II) would argue against this possibility. Finally, Zn(II) per se is not the source of inhibition with ATPαSB since it is a good activator in the reactions with ATP as substrate. Therefore, the simplest explanation for the inability to observe the reversal of stereospecificity of ATPαS is that the metal ion is not bound to the α-P at a rate-determining step during catalysis.

The results of the experiments with Zn(II) are similar to those from similar experiments on yeast Phe-tRNA synthetase in which reversal of stereospecificity was obtained with the ATPβS isomers but not with the ATPαS isomers (Connolly et al., 1980). ATPαSB was not a substrate with either Mg(II) or Co(II) (Connolly et al., 1980). As shown in Table IV with the Val-tRNA synthetase reversal of specificity in the exchange reaction with ATPβS as substrate is apparent; however, quantitatively these results may be distorted by the relatively fast interchange reaction (Rossomando et al., 1979).

For Met-tRNA synthetase from *E. coli* and for yeast Phe-tRNA synthetase, the βγ chelate of ATP is most likely the active metal–ATP complex. Similar evidence has been presented that DNA polymerase I (Burgers & Eckstein, 1979) and DNA-dependent RNA polymerase (Armstrong et al., 1979) from *E. coli* utilize the βγ chelate of ATP. These results are to be contrasted to those with kinases in which all patterns of reversal of stereospecificity of thio analogues have been observed: for yeast hexokinase only the β-S analogue shows reversal (Jaffe & Cohn, 1979a), for 3-phosphoglycerate kinase only the α-S analogue shows reversal (Jaffe & Cohn, 1979b), and for creatine kinase (Burgers & Eckstein, 1980) and pyruvate kinase (Jaffe & Cohn, 1979b) both α- and β-S analogues show reversal. Other adenylyl transferring enzymes should be investigated to establish the generalization that the βγ chelate of ATP is always the active metal–nucleotide complex for this class of enzymes.

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