A Novel α-Proton Exchange Reaction Catalyzed by *Escherichia coli* Methionyl-tRNA Synthetase†

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ABSTRACT: The *Escherichia coli* truncated methionyl-tRNA synthetase (ΔMTS) was shown to catalyze α-carbon hydrogen–deuterium exchange of L-selenomethionine, L-methionine, L-ethionine, and L-norleucine in the presence of deuterium oxide. The rate of α-proton exchange for L-methionine was shown to be linear with respect to ΔMTS concentration. The exchange reaction showed saturation kinetics with apparent K_m values of 21 and 4 mM in the absence and presence of saturating adenosine concentrations, respectively. As expected, ΔMTS did not catalyze α-proton exchange of D-methionine since the enzyme has been shown to be specific for L-amino acids. In the absence of enzyme or in the presence of an equivalent concentration of Zn^{2+}, no hydrogen–deuterium exchange was detected. The exchange reaction was not observed with L-methioninol, an analogue of L-methionine lacking the carboxylate group. These results suggest that the α-carboxylate group is a requirement for the ΔMTS-catalyzed exchange reaction. The *E. coli* methionyl-tRNA synthetase (MTS) has previously been shown to be a zinc metalloprotein [Posorske, L. H., Cohn, M., Yanagisawa, N., & Auld, D. S. (1979) *Biochim. Biophys. Acta 576*, 128]. On the basis of the structural and mechanistic information available on MTS, we propose that the enzyme-bound zinc coordinates the carboxylate of the amino acid, while a base on the enzyme is responsible for exchange of the α-proton. The role of the enzyme-bound metal is to render the α-proton more acidic through coordination of the carboxylate group. This proposal suggests that the enzyme-bound zinc may have a catalytic role as well as a possible structural role in MTS. However, the role of this exchange reaction in amino acid activation and discrimination remains unknown.

Aminoacyl-tRNA synthetases are responsible for activation of their cognate amino acid with ATP via an aminoacyl-adenylate and transfer of the activated amino acid 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 the genetic code in translation.

$$\text{ATP} + \text{AA} \rightleftarrows \text{E·AMP–AA} + \text{PP}_i \quad (1)$$

$$\text{E·AMP–AA} + \text{tRNA} \rightarrow \text{E·AA–tRNA} + \text{AMP} \quad (2)$$

The *Escherichia coli* methionyl-tRNA synthetase is a member of a family of aminoacyl-tRNA synthetases which have been demonstrated to be zinc metalloproteins (Posorske et al., 1979; Mayaux & Blanquet, 1981). The native *E. coli* methionyl-tRNA synthetase (MTS)[1] is a dimeric enzyme with an apparent subunit molecular weight of 76K (Barker et al., 1982). The dimeric protein contains one tightly bound zinc atom per subunit (Posorske et al., 1979). In addition, two ATP binding sites have been found for the native protein, although the role of the second site is unknown (Fayat & Waller, 1974). Mild proteolysis with trypsin has been shown to produce a fully active monomeric fragment with an apparent molecular weight of 64K containing one tightly bound zinc per monomer and a single ATP binding site, it being the catalytic site (Cassio & Waller, 1974; Fayat & Waller, 1974; Posorske et al., 1979). The active trypsin fragment is produced by removal of about 130 amino acid residues from the carboxy terminus of the native protein. A crystallographic structure of the trypsin-modified MTS·ATP complex has located the enzyme-bound zinc near the aminoacyladenylate site (Brunie et al., 1987). From the crystal structure, the best candidates for zinc ligands are His28, Thr28, Asp83, His95, and possibly a water molecule (Brunie et al., 1987). However, knowledge of the exact metal coordination ligands in MTS will have to await further refinement of the crystallographic structure. In the absence of bound ATP at the aminoacyladenylate site, the conformation of the Zn^{2+} binding loop was found to be significantly different, prohibiting identification of the Zn^{2+} ligands (Brunie et al., 1990). Knowledge of the nature and number of Zn^{2+} ligands in the various MTS–substrate complexes is essential for understanding the role of zinc, catalytic or structural, in this important enzyme. It has been proposed that catalytic zinc atoms are coordinated to at least one H_2O molecule, whereas structural zinc atoms are coordinated only to protein ligands (Vallee & Auld, 1990). Zinc has been shown to be an absolute requirement for both MTS-catalyzed $tRNA^{Met}$ aminoacylation and ATP–pyrophosphate exchange activities (Mayaux et al., 1982). The zinc chelator 1,10-phenanthroline was found to inhibit the $tRNA^{Met}$ aminoacylation activity of MTS. However, the ATP–pyrophosphate exchange activity was found to be insensitive to the zinc chelator. On the basis of these results, the enzyme-bound zinc was proposed either to be part of the tRNA binding site or to play a structural role in maintaining the native conformation of the protein (Mayaux et al., 1982).

A truncated form of the *E. coli* methionyl-tRNA synthetase (ΔMTS) having a molecular weight of 66K, representing a slightly larger polypeptide than the trypsin fragment, has been

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[1] Abbreviations: MTS, methionyl-tRNA synthetase; ΔMTS, truncated methionyl-tRNA synthetase; DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate; GC/MS, gas chromatography/mass spectroscopy; Tris-d_{11}, tris(hydroxymethyl)aminomethane-d_{11}.

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cloned (Barker et al., 1982). ΔMTS has been shown be a monomer in solution and to contain only one MgATP binding site, it being the catalytic site (Rosevear, 1988). Kinetic properties of ΔMTS have been shown to be identical with those of the trypsin-modified and native enzyme (Rosevear, 1988). In an attempt to better understand the mechanism of amino acid activation and discrimination in MTS, we have initiated studies to determine the conformation and arrangement of substrates bound at the aminoacyladenylate site of ΔMTS. The ATP analogue Mg(α,β-methylene)ATP has been shown to bind to ΔMTS with an anti conformation about the adenine–ribose glycosidic bond (Williams & Rosevear, 1991). In addition, the conformation of the enzyme-bound nucleotide was found to be insensitive to the presence or the nature of amino acids bound at the aminoacyladenylate site. During the course of our studies on the conformation of amino acids bound at the aminoacyladenylate site of ΔMTS, we have discovered that ΔMTS catalyzes a novel α-proton exchange reaction with certain L-amino acids. This exchange reaction was observed with L-methionine, L-selenomethionine, L-ethionine, and L-norleucine, but not with D-methionine or L-methioninol, an amino acid derivative lacking the α-carboxylate group. It has been proposed that the enzyme-bound Zn^{2+}, located at the aminoacyladenylate site, is catalyzing the observed α-proton exchange in these amino acids. The role of the enzyme-bound metal or the exchange reaction in amino acid activation and/or discrimination remains unknown.

Materials and Methods

Materials. L-[*methyl*-^{14}C]Methionine and [^{32}P]pyrophosphate were purchased from New England Nuclear. Crude tRNA was purchased from Boehringer Mannheim. DEAE-Sepharose was obtained from Pharmacia. Tris-d_{11} and 99.9% deuterium oxide were obtained from MSD Isotopes. L-Methionine, D-methionine, L-selenomethionine, L-methioninol, L-norvaline, L-norleucine, L-ethionine, L-α-aminobutyrate, and Na_2Li_2(α,β-methylene)ATP were obtained from Sigma.

Enzyme Preparation. Truncated *E. coli* methionyl-tRNA synthetase was purified as previously described (Rosevear, 1988). By use of this procedure, ΔMTS was purified to apparent homogeneity as judged by SDS–polyacrylamide gel electrophoresis and by staining with Coomassie Brilliant Blue (Rosevear, 1988). Protein concentrations were estimated by Bradford (1976) or from the known extinction coefficients at 280 nm (Blanquet et al., 1973). The rate of methionyl-tRNA formation was assayed according to Barker et al. (1982). The methionine-dependent ATP–pyrophosphate exchange assay was performed as previously described (Heinrickson & Hartley, 1976; Fersht & Dingwall, 1979). Purified ΔMTS had specific activities of at least 2000 and 1000 units/mg in the aminoacylation and ATP–pyrophosphate exchange assays, respectively. For NMR experiments, the enzyme was first passed over a Chelex-100 column to remove trace paramagnetic impurities. Protein samples were concentrated and deuterated in an Amicon ultrafiltration cell by repeated ultrafiltration against NMR buffer (10 mM Tris-d_{11}, pH 7.5, containing 50 mM NaCl, 0.1 mM DTT, and 0.05 mM EDTA in ^2H_2O).

NMR Kinetic Measurements. Amino acids and analogues were prepared fresh in NMR buffer immediately before use, and the pH was adjusted with 0.1 mM NaOD or DCl if necessary. Hydrogen–deuterium exchange was monitored by observing the intensity of the α-proton resonance of the amino acid or analogue in the presence of ΔMTS at 37 °C. At the end of NMR experiments, ΔMTS was found to retain at least 90% of its original specific activity. Spectra were obtained at the indicated times by collecting 16 scans at 500 MHz with 8K data points, a sweep width of 5000 Hz, a 40° pulse, and a 3.5-s relaxation delay. Chemical shifts are relative to internal HDO. Spectra were processed by using an exponential multiplication of 7 Hz. Changes in the intensity of the α-proton resonance of the various amino acids or analogues as a function of time were measured by peak integration.

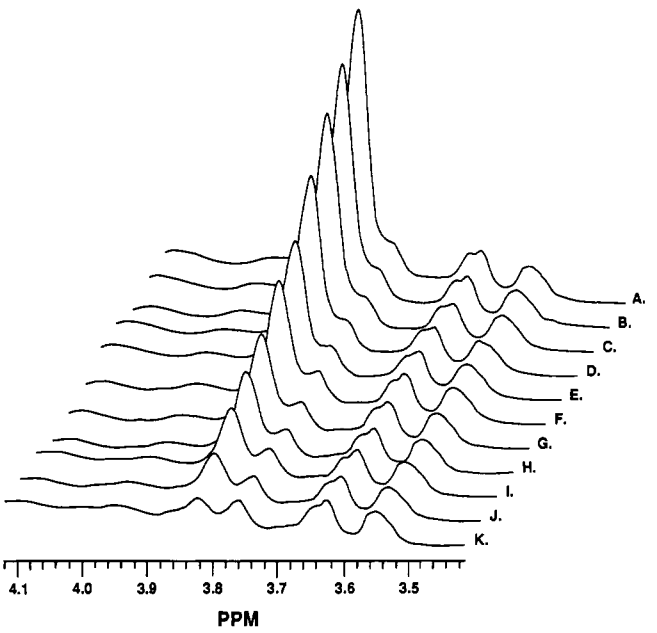

FIGURE 1: α-Proton exchange of L-selenomethionine in the presence of ΔMTS: Stacked plot showing the resonance of the α-proton of 25 mM L-selenomethionine in ^2H_2O at various times in the presence of 75 μM ΔMTS. Times are (A) 0, (B) 58, (C) 99, (D) 180, (E) 312, (F) 384, (G) 474, (H) 534, (I) 651, (J) 827, and (K) 1304 min. The sample contained 10 mM Tris-d_{11}, 50 mM NaCl, 0.1 mM DTT, and 0.05 mM EDTA at pH 7.5 in ^2H_2O. T = 37 °C. Spectra were acquired and processed as described under Materials and Methods.

Mass Spectroscopy. Amino acids were converted to the *N,O*-heptafluorobutyryl methyl esters. Methyl esterification was accomplished by using thionyl chloride/methanol (1:5) at room temperature for 45 min. Acylation was effected with heptafluorobutyric anhydride at 45 °C for 30 min. Reagents were removed in vacuo and the residues dissolved in chloroform. GC/MS analysis was performed on a Finnigan MAT INCOS 50 GC/MS under chemical irrigation conditions using methane as the reagent gas. Chromatographic separation was achieved on a 30-μm fused silica DB-5 capillary column operated from 100 to 200 °C at 4 °C/min.

Results

We report here that ΔMTS catalyzes the exchange of the α-proton of certain amino acids with those of the aqueous solvent. Figure 1 shows the ΔMTS-catalyzed time-dependent disappearance of the α-proton resonance of L-selenomethionine. The reaction was carried out in ^2H_2O buffer at pH 7.5 and the peak area of the α-proton of L-selenomethionine, 25 mM, monitored with time in the presence of 75 μM ΔMTS (Figures 1 and 2). At the end of the exchange reaction, deuteration of the α-proton was confirmed by GC/MS analysis (data not shown). In the absence of ΔMTS, no measurable α-proton exchange of L-selenomethionine was detected (Figure 2). The addition of 75 μM Zn^{2+} also failed to catalyze hydrogen–deuterium exchange of the α-proton of L-selenomethionine. Exchange of the α-proton of L-selenomethionine also occurred in the presence of Mg(α,β-methylene)ATP, a nonhydrolyzable analogue of MgATP (Figure 2). Mg(α,β-methylene)ATP has previously been shown to be a linear competitive inhibitor of

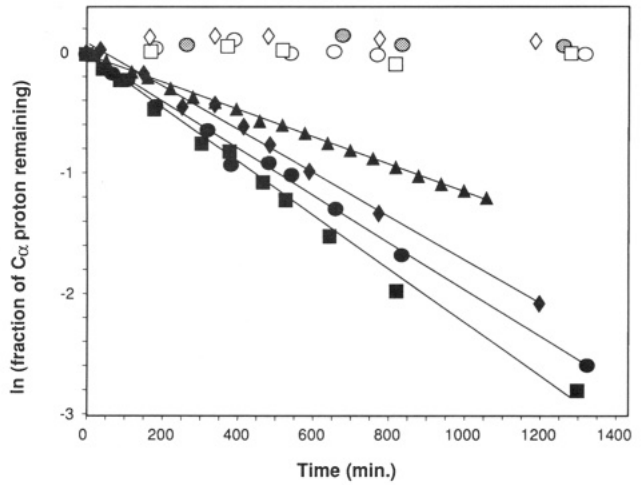

FIGURE 2: Rates of ΔMTS-catalyzed α-proton exchange for various amino acids. Rate constants are taken from the slope of the plot of ln (fraction of α-proton remaining at time *t*) versus time. Amino acids, 25 mM, were dissolved in 10 mM Tris-d_{11}, 50 mM NaCl, 0.1 mM DTT, and 0.05 mM EDTA at pH 7.5 in ^2H_2O: L-methionine (○); L-methionine + ΔMTS (●); D-methionine + ΔMTS (stippled circle); L-methionine + Mg(α,β-methylene)ATP + ΔMTS (▲); L-selenomethionine + ΔMTS (■); L-selenomethionine (□); L-ethionine + ΔMTS (♦); L-ethionine (◇). Conditions were as described in Figure 1.

Table I: ΔMTS-Catalyzed Exchange of the α-Hydrogen of Amino Acids and Amino Acid Analogues

substrate	k_{ex} ($\times 10^{-3}$ min^{-1} mg^{-1})[a]	substrate	k_{ex} ($\times 10^{-3}$ min^{-1} mg^{-1})[a]
L-methionine	1.00	L-selenomethionine	1.15
L-methionine + Mg(α,β-methylene)-ATP	0.59	L-ethionine	0.93
		L-norleucine	0.16
		L-norvaline	0[b]
D-methionine	0[b]	L-α-aminobutyrate	0[b]
L-methioninol	0[b]		

[a] α-Carbon hydrogen–deuterium exchange rates measured at 25 mM substrate and 75 μM ΔMTS at 37 °C as described under Materials and Methods. [b] No measurable α-hydrogen–deuterium exchange, <5%, detectable after 96 h at 37 °C. Estimation of the error was on the basis of the observed reproducibility of the peak integral in individual experiments.

MgATP in the ΔMTS-catalyzed ATP–pyrophosphate exchange reaction (Williams & Rosevear, 1991). Methionyl-tRNA synthetase was also found to catalyze hydrogen–deuterium exchange of the α-proton of L-methionine, L-ethionine, and L-norleucine (Figure 2; Table I). At the end of the exchange reactions, deuteration of the α-proton of all amino acids was confirmed by GC/MS. All four amino acids, L-selenomethionine, L-methionine, L-ethionine, and L-norleucine, have been shown to be substrates for MTS and are at least partially transferred to $tRNA^{Met}$ (Olds & Jones, 1977; Fersht & Dingwall, 1979). As expected, ΔMTS did not catalyze α-proton exchange of D-methionine (Figure 2), since the enzyme has been shown to be specific for L-amino acids (Olds & Jones, 1977). ΔMTS also failed to catalyze α-proton exchange of L-norvaline, and L-α-aminobutyrate under conditions of the NMR experiment. Both L-norvaline and L-α-aminobutyrate have been shown to be poor substrates in the ATP–pyrophosphate exchange assay catalyzed by *Bacillus stearothermophilus* MTS (Fersht & Dingwall, 1979). Saturation kinetics were not observed in the ATP–pyrophosphate exchange reaction for either of these amino acids within the accessible concentration range.

Rate constants for the α-proton exchange of L-methionine, 25 mM, were linear with respect to ΔMTS concentrations up

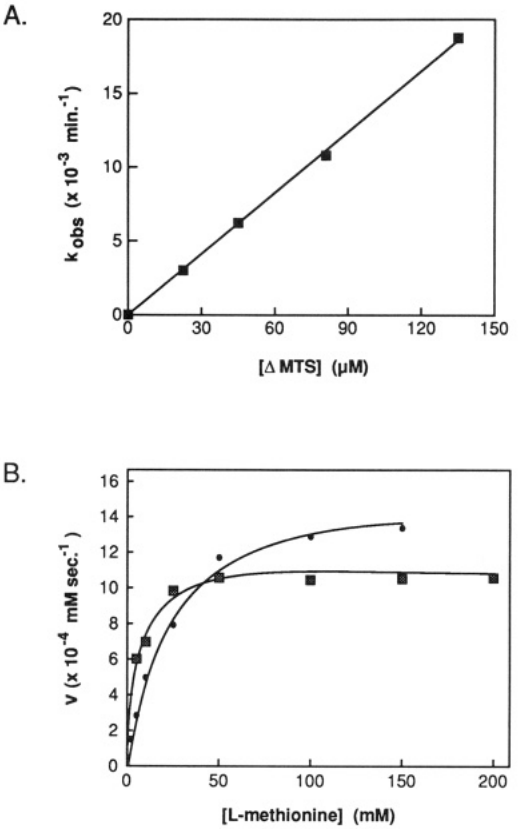

FIGURE 3: Characterization of L-methionine α-proton exchange catalyzed by ΔMTS. The dependence of the pseudo-first-order rate constant for exchange on the concentration of ΔMTS (A). Saturation kinetics for the α-proton exchange of L-methionine in the absence (●) and in the presence of a saturating concentration of adenosine (stippled square) (B). Experimental conditions are as described under Materials and Methods.

to 135 μM, as expected for an enzyme-catalyzed reaction (Figure 3A). Saturation kinetics were observed for the α-proton exchange of L-methionine in the absence and presence of a saturating concentration of adenosine (Figure 3B). The apparent K_m values for L-methionine in the presence of enzyme alone and enzyme plus a saturating concentration of adenosine were 21 and 4 mM, respectively (Figure 3B). These values are significantly higher than K_d values determined by equilibrium dialysis, 75 and 16 μM, for L-methionine in the absence and presence of adenosine, respectively (Blanquet et al., 1975). Since the K_m values for exchange were found to be several orders of magnitude greater than the K_d values, at least one additional enzyme–substrate complex must be required for the α-proton exchange reaction. However, the presence of adenosine decreases considerably the K_d for L-methionine and the K_m for L-methionine in the α-proton exchange reaction. The magnitude of these couplings, i.e., change in K_m or K_d of one ligand upon the binding of another ligand at the amino-acyladenylate site, was similar, 4.8 and 4.7, respectively. This relative difference in the binding and Michaelis constants translates to the other amino acids (data not shown), and hence the enzyme could not be saturated with the more weakly binding amino acids. Therefore, k_{ex} values for the various amino acids were compared at a fixed amino acid concentration, 25 mM (Table I). Exchange rates for L-methionine, L-ethionine, and L-norleucine (Table I) were found to parallel the dissociation constants determined for these amino acids under similar conditions (Rosevear, 1988). Thus, differences in the experimentally determined k_{ex} values likely reflect differences in the Michaelis constants of the various amino acids in the α-proton exchange reaction catalyzed by ΔMTS.

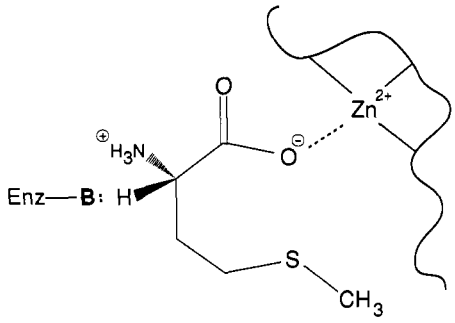

FIGURE 4: Hypothetical ΔMTS·L-methionine complex showing the proposed coordination of the enzyme-bound Zn^{2+} with the carboxylate of L-methionine and the enzymic base responsible for α-proton exchange.

The k_{cat} value for the exchange of hydrogen for deuterium at the α-proton of L-methionine was calculated to be 7×10^{-3} s^{-1} at pH 7.5 and 37 °C. Although a direct comparison of the enzyme's catalytic efficiency with the α-proton exchange reaction is not feasible, the k_{cat} for aminoacylation of $tRNA^{Met}$ with L-methionine at 30 °C has been found to be approximately 2–3 s^{-1} (Rosevear, 1988; Williams & Rosevear, 1991).

ΔMTS was found not to catalyze α-proton exchange of L-methioninol, an analogue of methionine lacking the carboxylate group (Figure 2, Table I). L-Methioninol has been shown to be a competitive inhibitor of L-methionine in both the aminoacylation and the ATP–pyrophosphate exchange reactions catalyzed by MTS (Fayat et al., 1977). This observation underscores the importance of the carboxyl group in the α-proton exchange reaction. Thus, ΔMTS catalyzes α-proton exchange of only L-amino acids having an α-carboxylate group.

Discussion

The truncated *E. coli* methionyl-tRNA synthetase was found to catalyze hydrogen–deuterium exchange of the α-proton of L-methionine, L-selenomethionine, L-ethionine, and L-norleucine (Figure 2, Table I). All of these amino acids have previously been shown to be substrates in the aminoacylation and ATP–pyrophosphate exchange reactions catalyzed by MTS. L-Methionine, L-selenomethionine, and L-ethionine have also been shown to be transferred to $tRNA^{Met}$ (Fersht & Dingwall, 1979; Olds & Jones, 1977). L-Norleucine, which is nominally isosteric with the cognate amino acid L-methionine, has been shown to be activated by MTS and both transferred to cognate $tRNA^{Met}$ and presumably edited (Fersht & Dingwall, 1979). No α-proton exchange was observed with either L-norvaline or α-aminobutyrate (Table I). Both amino acids are sterically smaller than methionine and are poorly activated by MTS (Fersht & Dingwall, 1979). As expected, ΔMTS did not catalyze α-proton exchange of D-methionine (Table I), since the enzyme has previously been shown to be specific for L-amino acids (Old & Jones, 1977). Thus, ΔMTS catalyzes an α-proton exchange reaction having the identical amino acid specificity as exhibited in the aminoacylation reaction. Although the apparent K_m for methionine, 21 mM, was found to be several orders of magnitude greater than the experimentally measured K_d (Rosevear, 1988), both reactions exhibited identical couplings upon addition of nucleotide.

On the basis of the structural and mechanistic information available on MTS, the scheme shown in Figure 4 provides a hypothesis to account for MTS-catalyzed exchange of the α-proton of L-selenomethionine, L-methionine, L-ethionine, and L-norleucine. Since the enzymic zinc has been located at the aminoacyladenylate site in ΔMTS (Brunie et al., 1987, 1990) and the α-carboxylate group is required for α-carbon hydrogen–deuterium exchange, we propose that the enzyme-bound zinc coordinates the α-carboxylate while a base on the enzyme is responsible for the exchange of the α-proton (Figure 4). The enzyme-bound metal will render the α-proton more acidic through coordination of the carboxylate group. Figure 4 has been drawn with the carboxylate directly coordinated to the metal ion, although it is possible that a water molecule might intervene. Polydentate metal complexes of carboxylic acids and amino acids are known to undergo exchange of their α-methylene or α-methine protons (Ama et al., 1980; Terrill & Reilley, 1988). The major factor determining the α-hydrogen exchange rate in these systems was found to be the chelate ring strain of the poly(aminocarboxylato) complex (Ama et al., 1980). An enzymatic mechanism for α-proton exchange has been proposed for the racemization of mandelic acid by mandelate racemase (Kenyon & Hegeman, 1970; Fee et al., 1974a,b). Mandelate racemase has been shown to have an absolute requirement for divalent cations. The divalent cation coordinates the carboxylate of mandelic acid and withdraws electrons, thereby facilitating abstraction of the α-proton (Fee et al., 1974a,b).

Methionine has been shown to be capable of metal coordination through its amino, carboxylate, and the thioether groups (Lenz & Martell, 1964; McAuliffe et al., 1966). Only the carboxylate group of the amino acid is shown coordinating the enzyme-bound zinc since the thioether group is not an absolute requirement for proton exchange and it has been proposed that an acidic residue of the protein specifically interacts with the amino group of L-methionine (Brunie et al., 1990). In the tyrosyl-tRNA synthetase, the tyrosine is known to be bound by interactions between its amino group and an acidic residue on the protein (Blow et al., 1983). However, bidentate coordination of L-methionine, L-ethionine, and L-selenomethionine cannot be unequivocally ruled out. Preliminary nuclear Overhauser effect spectra suggest that the α-proton is within 5 Å of the δCH_3 of enzyme-bound L-selenomethionine.

In summary, we have demonstrated that the *E. coli* methionyl-tRNA synthetase catalyzes a novel α-proton exchange reaction with certain L-amino acids. It has been proposed that this reaction is facilitated through coordination of the α-carboxylate group of the amino acid with the enzyme-bound zinc. This proposal suggests that the enzyme-bound zinc may have a catalytic role, as well as a structural role, in amino acid activation and/or discrimination, although the role of the exchange reaction in these processes remains to be defined. Deuterium exchange experiments with L-isoleucine and L-leucine in the presence of *E. coli* isoleucyl-tRNA synthetase failed to detect enzyme-catalyzed α-proton exchange with this aminoacyl-tRNA synthetase. The *E. coli* isoleucyl- and methionyl-tRNA synthetases have been suggested to have similar secondary structural motifs including the position of the catalytic sequence, His-hydrophobic-Gly-[His or Asn], but with the insertion of polypeptides of varying size into adjacent regions (Burbaum et al., 1990). Therefore, MTS-catalyzed hydrogen–deuterium exchange may be specific for this aminoacyl-tRNA synthetase and developed as a consequence of the nature of its cognate amino acid, L-methionine. Studies are currently in progress to more fully characterize this unique α-proton exchange reaction and to further define the role of the enzyme-bound zinc in this process.

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Site-Directed Mutagenesis Studies with *Eco*RV Restriction Endonuclease To Identify Regions Involved in Recognition and Catalysis[†,‡]

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ABSTRACT: Guided by the X-ray structure analysis of a crystalline *Eco*RV–d(GGGATATCCC) complex (Winkler, in preparation), we have begun to identify functionally important amino acid residues of *Eco*RV. We show here that Asn70, Asp74, Ser183, Asn185, Thr186, and Asn188 are most likely involved in the binding and/or cleavage of the DNA, because their conservative substitution leads to mutants of no or strongly reduced activity. In addition, C-terminal amino acid residues of *Eco*RV seem to be important for its activity, since their deletion inactivates the enzyme. Following the identification of three functionally important regions, we have inspected the sequences of other restriction and modification enzymes for homologous regions. It was found that two restriction enzymes that recognize similar sequences as *Eco*RV (*Dpn*II and *Hin*cII), as well as two modification enzymes (M·*Dpn*II and, in a less apparent form, M·*Eco*RV), have the sequence motif -SerGlyXXXAsnIleXSer- in common, which in *Eco*RV contains the essential Ser183 and Asn188 residues. Furthermore, the C-terminal region, shown to be essential for *Eco*RV, is highly homologous to a similar region in the restriction endonuclease *Sma*I. On the basis of these findings we propose that these restriction enzymes and to a certain extent also some of their corresponding modification enzymes interact with DNA in a similar manner.

Type II restriction endonucleases catalyze the site-specific cleavage of double-stranded DNA [reviews: Modrich and Roberts (1982), Malcolm and Snousnou (1987), Bennett and Halford (1989), and Pingoud et al. (1990)]. Over 1200 different restriction enzymes have been discovered in a wide variety of prokaryotes (Roberts, 1990), among them many isoschizomers which recognize the same DNA sequence. Altogether, about 130 different specificities have been described.

*Eco*RV is one of the best characterized restriction enzymes [review: Luke et al. (1987)]. Its sequence is known (Bougueleret et al., 1984). It has been purified to homogeneity (d'Arcy et al., 1985) from an overproducing strain (Bou-

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[‡] Crystallographic coordinates for the complex between *Eco*RV and a noncognate DNA fragment have been submitted to the Brookhaven Protein Data Bank.

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