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The Glutamyl-tRNA Synthetase of *Escherichia coli* Contains One Atom of Zinc Essential for Its Native Conformation and Its Catalytic Activity†

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ABSTRACT: The glutamyl-tRNA synthetase of *Escherichia coli* contains one atom of zinc. This metal ion is strongly bound, as it is not removed by 8 M urea. Slow removal of the zinc at 4 °C in the presence of the specific chelating agent, 1,10-phenanthroline, is proportional to the loss of aminoacylation activity and to the presence of a more open conformer of the enzyme. This conformer migrates more slowly than the native enzyme during gel electrophoresis under nondenaturing conditions and binds $tRNA^{Glu}$. Infrared spectroscopy measurements show that it differs from the native enzyme by a lower α-helix content and a higher proportion of β-sheet and unordered structures. ATP protects the enzyme against 1,10-phenanthroline-mediated zinc removal, suggesting that the zinc-binding region is closely associated with the catalytic site. Additional support for this conclusion comes from the presence of zinc in the 27-kDa N-terminal half of the enzyme and in a 10-kDa fragment. The latter is homologous to the tRNA acceptor helix binding domain of *E. coli* glutaminyl-tRNA synthetase. The presence of the conserved CYC motif in this domain of the zinc-containing glutamyl-tRNA synthetases of *E. coli* and *Bacillus subtilis*, and its absence in that of *Thermus thermophilus* and the *E. coli* glutaminyl-tRNA synthetase which do not contain zinc, suggest that the cysteines of this motif and the C- and H-rich $^{125}CRHSHEHHX_5C^{138}$ segment present in the 10-kDa zinc-binding fragment are involved in zinc binding by the glutamyl-tRNA synthetase of *E. coli*.

The aminoacyl-tRNA synthetases (aaRSs)[1] catalyze a key step in protein biosynthesis. Their specific interactions with their tRNA substrates define the genetic code. Some of them have additional functions in which they interact with other nucleic acids: a transcriptional operator for the AlaRS of *Escherichia coli* (Putney & Schimmel, 1981), a translational operator for the ThrRS of *E. coli* (Springer *et al.*, 1989), and group I introns for two fungal mitochondrial aminoacyl-tRNA synthetases [Kittle *et al.*, 1991; for a review, see Lapointe and Giegé (1991)].

Comparison of the primary structures of aaRSs specific for each of the 20 amino acids involved in protein biosynthesis led to the discovery of their partition into two classes characterized by short amino acid sequence motifs (Eriani *et al.*, 1990). The three-dimensional structures of some members of class I, TyrRS, MetRS, and GlnRS, are similar and differ radically from those of class II, such as SerRS and AspRS (Cusack *et al.*, 1990; Ruff *et al.*, 1991). The presence of zinc has been reported in several aminoacyl-tRNA synthetases from class I: MetRS from *E. coli* (Posorske *et al.*, 1979), *Thermus thermophilus* HB8 (Kohda *et al.*, 1984; Nureki et al., 1991), and sheep liver (Lazard *et al.*, 1985); IleRS from *E. coli*; ValRS from *T. thermophilus* HB8 (Mayaux & Blanquet, 1981; Kohda *et al.*, 1984), and TrpRS from bovine pancreas (Kisselev *et al.*, 1981). It is also present in a member of class II, the *E. coli* AlaRS (Miller *et al.*, 1991). Its absence has been reported for one class I enzyme, *E. coli* TyrRS (Mayaux & Blanquet, 1981); one class II enzyme, *E. coli* PheRS, contains no strongly bound zinc, but it can bind eight atoms of zinc with a relatively low affinity (Mayaux & Blanquet, 1981). These data suggest that the presence of zinc is conserved among aminoacyl-tRNA synthetases specific for the same amino acid, but is not linked to a structural class.

So far, the position of zinc has been determined by x-ray crystallography only for the tryptic fragment of *E. coli* MetRS (Brunie *et al.*, 1990). In this enzyme, zinc has been located near the active site. Moreover, the existence of structures analogous to zinc fingers has been proposed from the presence of zinc in domains containing amino acid sequences similar to those of characterized zinc fingers and from the loss of either the activation or the transfer reaction of aaRSs whose proposed zinc-binding cysteine residues had been conservatively replaced. Such structures have been proposed for two class I aaRSs, *T. thermophilus* and *E. coli* MetRSs (Nureki *et al.*, 1991; Landro & Schimmel, 1993), and for a class II aaRS, *E. coli* AlaRS (Miller *et al.*, 1991). The zinc finger-like sequence of *T. thermophilus* MetRS ($^{127}CX_2CX_{13}CX_2H$) is similar to a typical zinc finger motif for eukaryotic DNA-binding proteins (Nureki *et al.*, 1991), whereas that of *E. coli* AlaRS ($CX_2CX_6HX_2H$) resembles that of a retroviral nucleic acid binding protein (Miller *et al.*, 1991) and was shown to be important for tRNA recognition (Miller & Schimmel, 1992).

We report here on the zinc content of two members of class I, whose primary structures indicate a strong evolutionary linkage: glutamyl-tRNA synthetase (GluRS) and glutaminyl-tRNA synthetase (GlnRS) of *E. coli* (Breton *et al.*, 1990). GlnRS contains no zinc, whereas GluRS contains one atom

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[1] Abbreviations: aaRS, aminoacyl-tRNA synthetase; XyzRS, aminoacyl-tRNA synthetase specific for the amino acid whose standard abbreviation is Xyz; OP, 1,10-phenanthroline; SDS–PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate; X, any amino acid.

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of zinc whose removal triggers a conformational change in the enzyme and the loss of its aminoacylation activity. This difference between these two enzymes closely linked in their evolution, together with the presence of zinc in GluRS from *Bacillus subtilis* reported here and its absence in that of *Thermus thermophilus* (Nureki *et al.*, 1993), indicate that the zinc-binding sites may have been gained and lost frequently in the evolution of these aminoacyl-tRNA synthetases, possibly to modulate their interactions with the acceptor stems of their tRNA substrates.

MATERIALS AND METHODS

1,10-Phenanthroline (OP), sodium *N*-(2-hydroxyethyl)-piperazine-*N'*-2-ethanesulfonic acid (sodium Hepes), DL-dithiothreitol (DTT), β-mercaptoethanol, adenosine triphosphate (ATP), bovine serum albumin (BSA), thermolysin from *Bacillus thermoproteolyticus rokko*, and the endoproteinase Glu-C from *Staphylococcus aureus* strain V8 were purchased from Sigma Chemical; magnesium chloride, disodium ethylenediaminetetraacetate (EDTA), and standard solutions of Zn^{2+} for atomic absorption spectroscopy were from Fisher Scientific Company. Glycerol was obtained from Biopharm, trichloroacetic acid was from Anachemia, unfractionated tRNA and $tRNA_2^{Glu}$ from *E. coli* MRE600 were from Boehringer Mannheim, and uniformly labeled L-[^{14}C]-glutamate was from New England Nuclear. Materials for gel electrophoresis (molecular weight marker, acrylamide, bisacrylamide) and Chelex-100 were purchased from Bio-Rad, while dialysis membranes (MWCO 25 000, metal- and sulfur-free) were from Spectrum, Centricon-30 was from Amicon, and ultrapure urea was from Bethesda Research Laboratories. All solutions were prepared with metal-free deionized water obtained in two steps: reverse osmosis (Milli-RO 40, Millipore Co.) and then ion exchange (Barnstead NANO pure system). All experiments requiring metal-free conditions were carried out in plasticware soaked in 30% nitric acid (Stöcker *et al.*, 1988) for 1 day and rinsed extensively with metal-free water. Glutaminyl-tRNA synthetase from *E. coli* was kindly provided by Dr. D. Söll.

Purification and Characterization of E. coli GluRS and Its Fragments. GluRS was purified from the overproducing strain, *E. coli* DH5α (pLQ7612) (Brisson *et al.*, 1989), by a rapid procedure, as previously described (Lin *et al.*, 1992), with the following modification: 10 μM $ZnCl_2$ was added to the growth medium. The homogeneity of the purified enzyme was verified by electrophoresis on polyacrylamide gel in the presence of SDS (SDS–PAGE), after denaturation and reduction of the protein (Laemmli, 1970). GluRS was partially proteolyzed either with thermolysin, as previously described (Brisson *et al.*, 1989), or with the extracellular protease of *Staphylococcus aureus* strain V8 (Houmard & Drapeau, 1972). GluRS fragments were separated by anion-exchange chromatography on a Mono-Q HR5/5 FPLC column (Pharmacia), using an LKB 2150-003 HPLC pump with a titanium head. Protein concentrations were determined by the optical method of Warbourg and Christian (1942): [protein] (mg/mL) = $1.55A_{280nm} - 0.76A_{260nm}$. N-terminal sequences of GluRS fragments were determined by automatic Edman degradation performed on an Applied Biosystems Model 473A pulsed liquid protein sequencer at the "Service de Séquence de l'Est du Québec (Centre de recherche du CHUL)". The amount of protein in various bands stained with Coomassie blue, following gel electrophoresis, was determined with a Molecular Dynamics Model 300A computing densitometer.

Determination of the Metal Content of Several Aminoacyl-tRNA Synthetases. The metal content of *E. coli* GluRS was determined by X-ray fluorescence and by atomic absorption spectroscopy. X-ray fluorescence measurements were performed with a silicium detector and a 1024-channel analyzer calibrated with thin layers of zinc obtained by evaporation of a certified atomic absorption solution of zinc. The enzyme solution was excited with white radiation produced by an X-ray generator (Mo tube) operated at 10 mA and 40 kV. The amounts of zinc and other transition metals were calculated from the intensities of their Kα emission bands. Atomic absorption spectroscopy measurements were conducted with a Perkin-Elmer Model 2380 atomic absorption spectrometer at wavelengths of 213.9 for Zn, 285.2 for Mg, and 422.7 nm for Ca. Enzyme samples were washed several times in Centricon-30 with a metal-free buffer containing 5 mM sodium Hepes (pH 7.5) and 0.5 mM DTT. This buffer does not affect the determination of Zn by the methods described above. The buffer used for the last wash was taken as the control. In these analyses, the GluRS and GlnRS concentrations were about 4 and 15 μM, respectively, and the calibration curve was obtained with 0.05–1.0 μg of zinc/mL. At least two determinations were made for each sample. The zinc content of GluRS fragments was determined by atomic absorption in the presence of 4 M urea. The fragments eluted from the Mono-Q column in 8 M urea were diluted with 1 vol of water; for this experiment, zinc standards were made with equal volumes of metal-free water and elution buffer.

Protein Secondary Structure Estimation by Infrared Spectroscopy. Infrared spectra were recorded with a Bomen DA3-02 Fourier transform spectrophotometer with a narrow-band mercury–cadmium–telluride detector and a germanium-coated KBr beam splitter. One thousand interferograms were recorded with a maximal optical retardation of 0.5 cm, coadded, triangularly apodized, and Fourier transformed to yield a resolution of 2 cm^{-1}. GluRS treated with 1,10-phenanthroline was washed in Centricon-30 with 50 mM sodium Hepes (pH 7.2) until all of the 1,10-phenanthroline was removed, as indicated by low UV absorbance. About 20 μL of GluRS (50 mg/mL) in 50 mM sodium Hepes (pH 7.2) was used for each experiment. This buffer gives no detectable signal in this region of the infrared spectrum.

Transmission spectra were obtained at 20.0 ± 0.1 °C using a cell composed of two CaF_2 windows separated by a 6-μm spacer. Fourier deconvolution of the amide I region was done by the method of Griffiths and Pariente (1986), using a narrowing parameter, γ, of 5.5 and an apodization filter of 0.14.

Fluorescence Measurement of the GluRS·tRNA^{Glu} Interaction. The interaction between $tRNA^{Glu}$ and either native GluRS or the soluble form of zinc-depleted GluRS was studied by quenching of the enzyme fluorescence by $tRNA^{Glu}$ using a Shimadzu Model RF-540 recording spectrofluorophotometer (P/N 204-02900). The measurements were made at room temperature in 50 mM sodium Hepes (pH 7.2) and 2 mM $MgCl_2$. The fluorescence was excited at 290 nm and was measured at 350 nm to avoid Raman scattering of water molecules (Hara-Yokoyama et al., 1986). The concentration of either form of the enzyme was 0.1 μM.

Enzyme Assay. GluRS was assayed at 37 °C by measuring the rate of formation of [^{14}C]glutamyl-tRNA (Lin *et al.*, 1992). The final concentration of GluRS in the reaction mixture was 0.2 μg/mL, to ensure the initial velocity conditions.

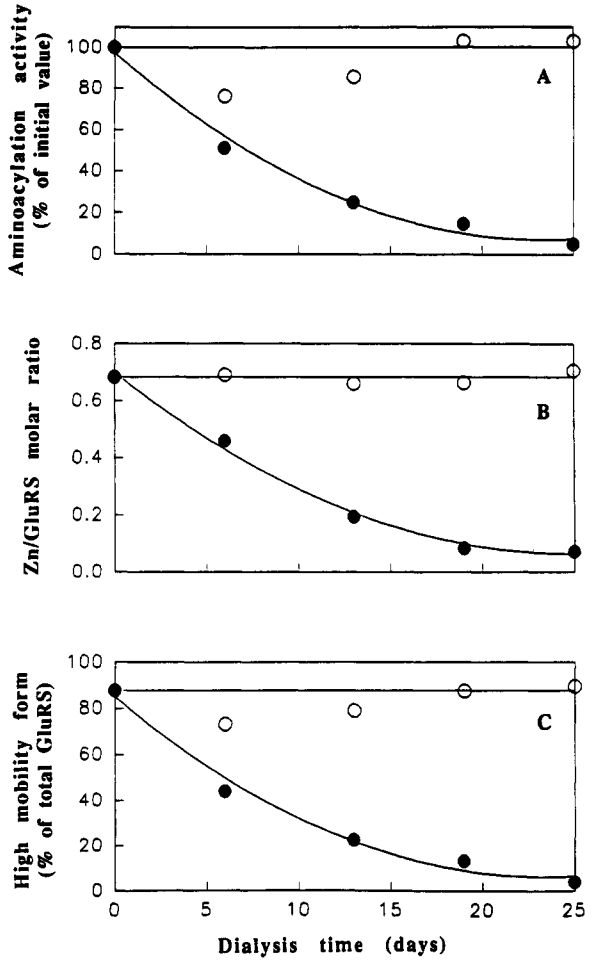

FIGURE 1: Influence of GluRS dialysis against 1,10-phenanthroline (OP) on its aminoacylation activity, zinc content, and electrophoretic mobility. GluRS samples (4 mg/mL, 2 mL each) were dialyzed at 4 °C against 50 mM sodium Hepes (pH 7.2), with(●) or without (○) 10 mM OP. The solutions were changed every day with Centricon-30 microconcentrators. At various times, aliquots were withdrawn for measurements of (A) the aminoacylation activity, (B) the zinc content, and (C) the percentage of the high mobility form in total GluRS (20–30 μg). The latter was determined by microdensitometry of Coomassie blue stained protein bands after gel electrophoresis (see Figure 3).

Gel Electrophoresis under Nondenaturing Conditions. For studies of protein conformation, a discontinuous buffer system including a 10% polyacrylamide separating gel was prepared as described by Ausubel *et al.* (1987). Samples of 10–30 μg of protein were applied to each well of a 1.5 mm thick slab of gel (10 cm wide, 6 cm long, 10 wells comb). Electrophoresis was conducted at room temperature (25 °C) for 2 h at a current of 25 mA. Protein bands were stained with Coomassie brilliant blue.

RESULTS

Metal Content of E. coli GluRS. X-ray fluorescence of GluRS revealed the presence of about 0.5 mol of zinc/mol of protein and the absence of significant amounts of Fe, Co, Ni, Cu, and Pb. The more precise method of atomic absorption spectroscopy yielded values of 0.6 ± 0.1 mol of zinc/mol of protein and trace amounts of Ca and Mg.

The chelating ion-exchange resin Chelex-100 binds zinc very strongly (Holmquist, 1988). In order to test whether zinc is tightly bound to GluRS, 10 mL of 0.25 mg/mL (4.6 μM) GluRS in 10 mM sodium Hepes (pH 7.5) was passed at room temperature through a Chelex-100 column (4 × 1 cm) at a rate of 0.3 mL/min. The zinc content of the eluted fractions was determined by atomic absorption. Passage of GluRS through this column did not remove its zinc (results not shown). As a control, a solution of 0.26 ppm zinc in the same buffer was passed through the column at the same flow rate; no zinc was detected in the eluted fractions. From this experiment, we conclude that the zinc atom is tightly bound to GluRS.

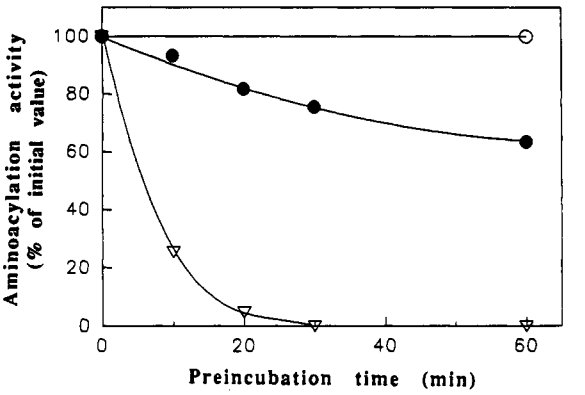

FIGURE 2: Influence of ATP on GluRS stability in the presence of OP. GluRS (0.2 mg/mL) was preincubated at 50 °C in 100 mM sodium Hepes (pH 7.2), 10% glycerol, and 20 mM β-mercaptoethanol without OP (○) with 10 mM OP (▽), and with 5 mM ATP and 10 mM OP (●). Aliquots were diluted 1000-fold in GluRS assay mixtures.

Zinc Removal Inactivates GluRS and Is Inhibited by ATP. 1,10-Phenanthroline (OP) is a strong chelator for zinc (Posorske *et al.*, 1979). GluRS samples (4 mg/mL) were dialyzed at 4 °C against 50 mM sodium Hepes (pH 7.2) with or without 10 mM OP. The solutions for dialysis were changed every day. Aliquots were withdrawn at various times for measurements of zinc content and aminoacylation activity and for electrophoretic analysis. Dialysis against 10 mM OP slowly decreased GluRS activity in parallel with its zinc content (Figure 1 A,B), with a half-life of 6 days. On the other hand, GluRS dialyzed without OP kept its activity and its zinc content. Other GluRS samples (4 mg/mL) dialyzed under the same conditions had no detectable catalytic activity nor zinc after 47 days in the presence of 10 mM OP. Therefore, zinc is essential for GluRS aminoacylation activity. In this context, the fact that dialysis of GluRS for 30 min at 37 °C against 10 mM EDTA, 100 mM sodium Hepes, and 10% glycerol does not affect its aminoacylation activity is another indication of the strong binding of zinc to the enzyme.

The rate of GluRS inactivation and zinc depletion in the presence of OP increases markedly with temperature (results not shown), indicating that the thermal motion of the enzyme makes the strongly bound zinc more accessible to the solvent. At 50 °C, the presence of 5 mM ATP strongly protects GluRS against OP-mediated inactivation, increasing its half-life from 5 min to more than 1 h (Figure 2), which suggests that the zinc-binding region is closely associated with the ATP-binding site.

Zinc Removal Triggers a Conformational Change in GluRS. Polyacrylamide gel electrophoresis under nondenaturing conditions shows that GluRS depleted of its zinc by a long incubation at 4 °C in the presence of OP is soluble and migrates more slowly than native GluRS, whereas GluRS depleted of zinc at 50 °C is no more soluble and stays as a precipitate at the top of the gel (Figure 3). SDS–PAGE of this precipitate reveals only one band at the level of 53 kDa, as for native GluRS. This result indicates that the OP-mediated removal of zinc at low temperature triggers a

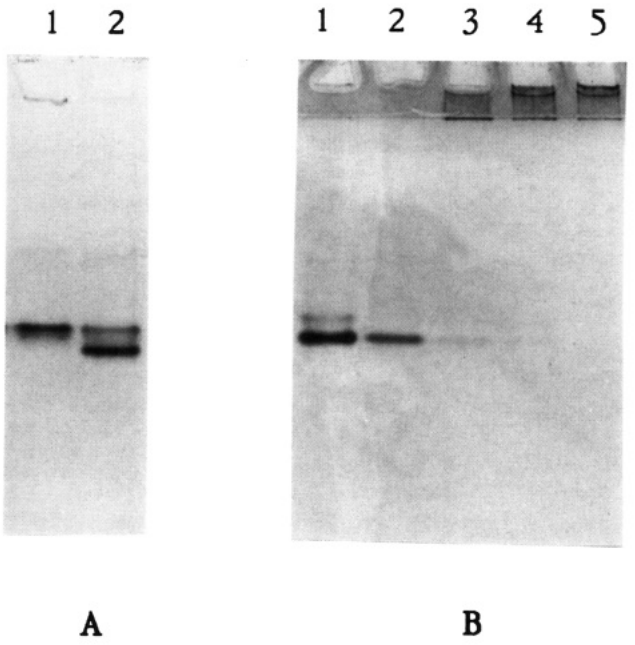

FIGURE 3: Polyacrylamide gel electrophoresis of native and OP-treated GluRS under nondenaturing conditions. (A) GluRS aliquots (4 mg/mL) were dialyzed at 4 °C against 100 mM sodium Hepes (pH 7.2), 10% glycerol, and 0.1 mM DTT containing either 10 mM OP (lane 1) or 0 mM OP (lane 2) for 47 days. (B) GluRS aliquots (0.2 mg/mL) were incubated at 50 °C in 100 mM sodium Hepes (pH 7.2), 10% glycerol, 20 mM β-mercaptoethanol, and 10 mM OP for various times: lane 1, native GluRS without treatment; lanes 2–5, 0, 10, 20, and 30 min of incubation, respectively.

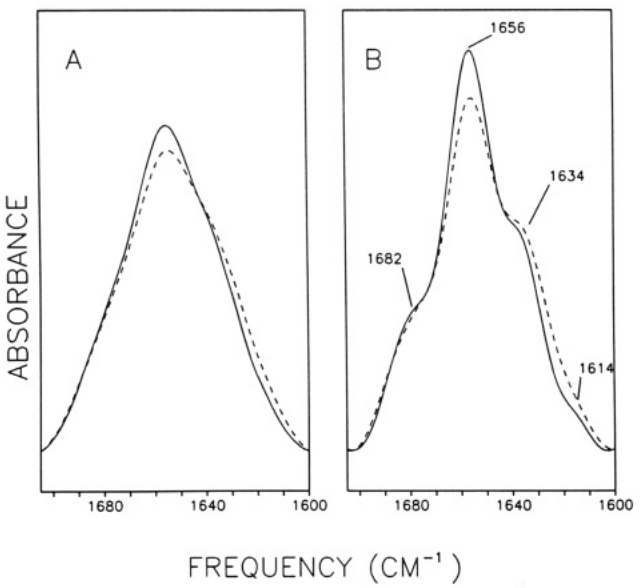

FIGURE 4: Water-subtracted infrared spectra in the amide I region for native GluRS (—) and the soluble form of zinc-depleted GluRS (---): (A) original and (B) Fourier deconvolved spectra.

conformational change of the enzyme and makes it much more sensitive to heat-inactivation, resulting in the formation of an insoluble precipitate. During incubation at 4 °C in the presence of OP, the percentage of the high mobility form (native) of GluRS is proportional to that of active and zinc-containing GluRS (Figure 1C). In spite of the conformational change of GluRS during zinc depletion, the enzyme still retained a strong affinity for $tRNA^{Glu}$, detected by fluorescence quenching (result not shown).

Infrared spectroscopy was also used to follow the conformational change induced by the removal of the zinc atom from the native protein. Spectra in the conformationally

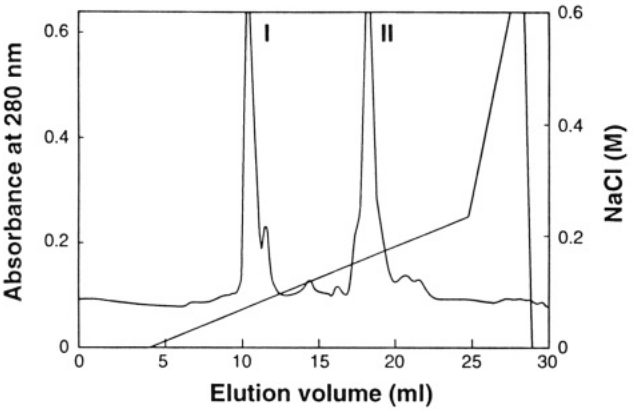

FIGURE 5: Separation of the N-terminal and C-terminal halves of GluRS by anion-exchange chromatography on a Mono-Q HR 5/5 column. GluRS (4.8 mg) in 2.8 mL of 100 mM Tris-HCl (pH 7.5), 10% glycerol, and 0.5 mM DTT was partially digested with thermolysin (25:1 w/w) for 1 h at 37 °C. This lysate was diluted 10 times with 8 M urea, 10% glycerol, 20 mM Tris-HCl (pH 7.9), and 20 mM β-mercaptoethanol and then applied to a Mono-Q column equilibrated with the same buffer. The elution was carried out at a flow rate of 0.5 mL/min with a 0–0.6 M NaCl gradient in the same buffer. The small peak eluted at 14 mL contains thermolysin, identified by SDS–PAGE; this protease contains zinc (Latt *et al.*, 1969).

sensitive amide I region of native GluRS and its zinc-depleted soluble form are shown in Figure 4A. These spectra were obtained after correction for the spectral contribution of the water bending vibration according to the method of Dousseau *et al.* (1989). As seen in this figure, the amide I is centered at 1656 cm^{-1}, showing that the predominant conformation of GluRS is α-helical (Byler & Susi, 1986; Surewicz & Mantsch, 1988; Dousseau & Pézolet, 1990). The spectra also reveal the presence of other types of secondary structure as shoulders are observed around 1680 and 1620 cm^{-1}. Fourier deconvolution of the spectrum (Figure 4B) reveals bands at 1634 and 1614 cm^{-1} assigned to extended and intermolecular β-sheet structures, respectively (Byler & Susi, 1986; Surewicz & Mantsch, 1988; Muga *et al.*, 1990; Carrier *et al.*, 1990; Pézolet *et al.*, 1992). The high-frequency component observed around 1680 cm^{-1} is associated with the presence of β-turns (Byler & Susi, 1986; Surewicz & Mantsch, 1988).

Figure 4 shows that the spectrum of the zinc-depleted GluRS is significantly different from that of the native protein. In both the original and Fourier deconvolved spectra of the zinc-depleted GluRS, the intensity of the 1656 cm^{-1} band is weaker while that of the 1614 and 1634 cm^{-1} features is stronger compared to the spectrum of the native protein. In order to quantify the results, the spectra in the amide I and amide II regions were analyzed using the method of Dousseau and Pézolet (1990). This method reveals that the conformation of native GluRS is composed of 41% α-helices and 28% β-sheet, while 31% of the secondary structure is unordered or contains turns. After zinc removal from the enzyme, the α-helix content is reduced to 33%, while the proportions of β-sheet and unordered structures increase to 33% and 34%, respectively. Therefore, these results show that the removal of the zinc atom from the enzyme results in a decrease in the amount of α-helical conformation at the expense of the extended conformation.

The Zinc-Binding Region Is in the N-Terminal Half of the GluRS. Partial proteolysis of GluRS by thermolysin yields two fragments whose apparent molecular masses were estimated as 31 and 29.5 kDa by SDS–PAGE (Brisson *et al.*, 1989). These two fragments correspond to residues of 1–237 and 238–471, and their molecular masses calculated from the known amino acid sequences are 27.3 and 26.5 kDa, respec-

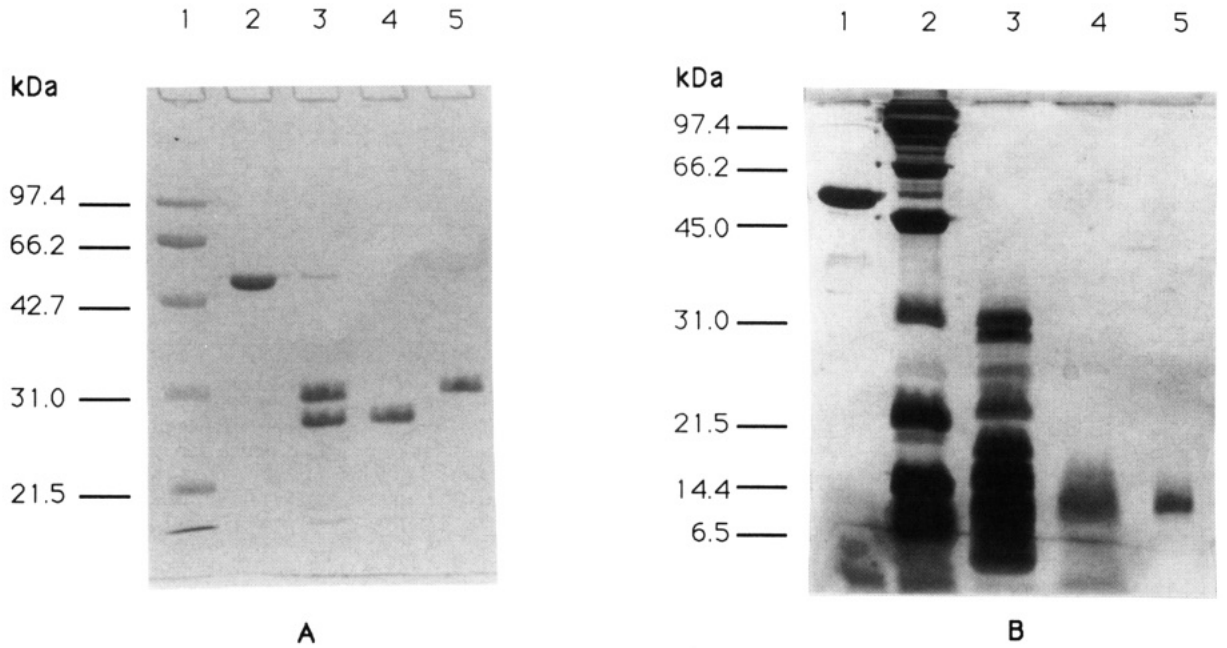

FIGURE 6: SDS–PAGE of proteolyzed GluRS and purified fragments. (A) After partial digestion with thermolysin: lane 1, MW markers; lane 2, GluRS; lane 3, total lysate (see Figure 5); lanes 4 and 5, central fractions of peak I (26.5 kDa) and peak II (27.3 kDa), respectively. (B) After extensive proteolysis with V8 protease: lane 1, native GluRS; lane 2, MW marker; lane 3, total lysate (see Figure 7); lanes 4 and 5, zinc-containing fractions, nos. 30 and 31 of Figure 7 (10 kDa).

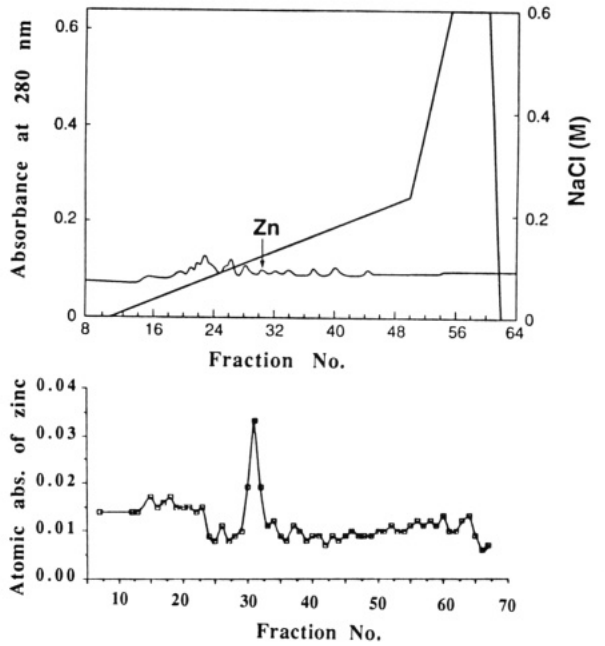

FIGURE 7: Separation of the peptides obtained by extensive proteolysis of GluRS with V8 protease and identification of the zinc-containing fractions. GluRS (1 mg) in 0.6 mL of 100 mM Tris-HCl (pH 7.5), 10% glycerol, and 0.5 mM DTT was digested extensively with V8 protease (5:1 w/w) for 1 h at 37 °C. The chromatography conditions were the same as those for Figure 5. For zinc measurements by atomic absorption, fraction aliquots were diluted with 1 vol of metal-free water. We verified that V8 protease contains no zinc.

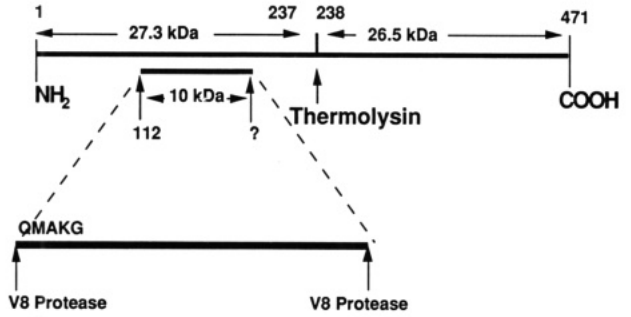

FIGURE 8: Location of the zinc-containing fragments in the *E. coli* GluRS primary structure.

tively. The product of partial digestion of GluRS with thermolysin was diluted 10-fold with 10% glycerol, 20 mM Tris-HCl, 8 M urea, and 20 mM β-mercaptoethanol and chromatographed on a Mono-Q FPLC column (Figure 5). The contents of the two well-resolved peaks were identified by SDS–PAGE (Figure 6A). Peak I eluted at about 0.078 M NaCl (Figure 5) and contained the 26.5-kDa fragment, corresponding to the carboxy-terminal half of GluRS, whereas peak II eluted at about 0.168 M NaCl and contained its amino-terminal half. Atomic absorption spectroscopy reveals the presence of 0.7 mol of zinc/mol of the amino-terminal half of GluRS and its absence in the carboxy-terminal half.

The products of a more extensive proteolysis of GluRS with V8 protease were separated by the same procedure. The eluted fractions were analyzed for their zinc content. A significant level of zinc was present only in a few consecutive fractions, corresponding to an A_{280nm} peak (Figure 7). These fractions contain a single polypeptide chain of about 10 kDa (Figure 6B). The sequence of five of its residues starting at its N-terminus was determined by sequential Edman degradation: it is QMAKG, which is identical to the ^{112}QMAKG^{116} present in the N-terminal half of GluRS (Figure 8).

We also determined the zinc contents of two enzymes homologous to *E. coli* GluRS: *Bacillus subtilis* GluRS contains 0.6 mol of zinc/mol of enzyme, but *E. coli* glutaminyl-tRNA synthetase contains no detectable amount of zinc. The N-terminal half of *E. coli* GluRS, where the zinc atom is bound, contains four of its five cysteines and four of its nine histidines in the short ^{98}C–^{138}C fragment (Brisson *et al.*, 1989). When the amino acid sequence of this segment is aligned with the corresponding segments of *B. subtilis* and *T. thermophilus* GluRSs and *E. coli* GlnRS, we see that the first two enzymes, which contain zinc, share the CYC motif aligned with ^{98}CYC of *E. coli* GluRS, whereas the last two enzymes, which do not contain zinc, do not contain cysteines at the corresponding

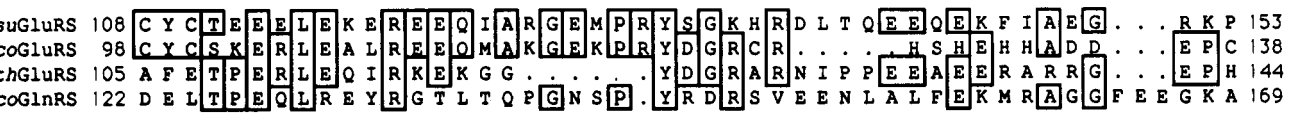

FIGURE 9: Alignment of the ^{98}C–^{138}C segment of *E. coli* GluRS (Breton *et al.*, 1986) with the homologous regions of *B. subtilis* (Breton *et al.*, 1990) and *T. thermophilus* GluRSs (Nureki *et al.*, 1992) and *E. coli* GlnRS (Yamao *et al.*, 1982).

positions (Figure 9). Considering that most residues participating in zinc binding by proteins interacting with nucleic acids are cysteines or histidines (Klug & Rhodes, 1987; Vallee & Auld, 1990), this correlation suggests that the two cysteines of the ^{98}CYC motif and C or H residues of ^{125}CRHSHEH-HX_5C^{138} may be involved in zinc binding by *E. coli* GluRS.

DISCUSSION

Our results show that *E. coli* GluRS contains zinc, whose removal causes the loss of aminoacylation activity and a conformational change. The presence of about 15% of the low mobility form in the "native" GluRS used in these studies (Figure 3, lane 2 of section A and lane 1 of section B) may be due to the fact that it was purified from an overproducing strain (Lin *et al.*, 1992) where intracellular zinc concentration may have been limiting. This form was never observed when the enzyme was purified from strains carrying only the chromosomal *gltX* gene, because the last purification step used was a preparative polyacrylamide gel electrophoresis, which would have excluded it (Kern *et al.*, 1979). In this context, the value of 0.6 ± 0.1 mol of zinc/mol of enzyme, obtained by atomic absorption, would correspond to a value of about 0.7 ± 0.1 mol of zinc/mol of the high mobility form of GluRS. Considering experimental uncertainties, this result is compatible with the presence of 1 zinc per GluRS.

Two different approaches indicate that the zinc-binding site is located in the ^{98}C–^{138}C segment: first, the presence of zinc in the N-terminal half of the enzyme and in a 10-kDa fragment starting at ^{112}Q, and secondly, the presence of two conserved cysteines in the zinc-containing *B. subtilis* GluRS, corresponding to ^{98}C and ^{100}C of *E. coli* GluRS, and their absence in the homologous parts of *T. thermophilus* GluRS and *E. coli* GlnRS which do not contain zinc (Figure 9). *E. coli* GluRS and GlnRS have a high level of amino acid sequence similarity in their N-terminal halves (Breton *et al.*, 1990), which correspond to GlnRS dinucleotide- and acceptor-binding domains (Rould *et al.*, 1989). The surprising stability of the zinc complexes with the N-terminal half of *E. coli* GluRS and with the 10-kDa fragment even in the presence of 8 M urea (Figures 7 and 8) is consistent with the long time required for zinc removal from the native enzyme by dialysis against the specific chelating agent, 1,10-phenanthroline.

The two sites where cleavage of *E. coli* GluRS by V8 protease generated this zinc-containing 10-kDa fragment are on the carboxy sides of ^{111}E (determined by sequencing; cf. Figure 8) and ^{193}E (from the size of this fragment) and correspond in the model made by aligning GluRS and GlnRS, respectively, to the extremity of the loop between helix E and β-strand 5 (^{136}T in GlnRS) in the acceptor-binding domain, and to the loop between helix G and β-strand 9 (^{223}G in GlnRS) at the beginning of the second half of the dinucleotide-binding domain (Breton *et al.*, 1990; Rould *et al.*, 1989). The amount of this fragment (which contains six glutamate residues, potential cleavage sites for the V8 protease, between ^{111}E and ^{193}E) recovered after relatively extensive proteolysis was sufficient for its detection by A_{280nm} in the column eluate (Figure 7) and by silver staining (Figure 6B) and sufficient for an unambiguous identification by Edman degradation of the five amino acids following ^{111}E (Figure 8). This result indicates that the GluRS fragment including the parts homologous to the β-strand 5–α-helix F–β-strand 6–β-strand 7–β-strand 8–α-helix G segment of GlnRS [according to the nomenclature of Rould *et al.* (1989)] stays folded in solution and therefore supports the correctness of GluRS model building based on GlnRS three-dimensional structure for its N-terminal half.

The inhibition by ATP of zinc removal from GluRS (Figure 2) is consistent with the fact that the zinc-containing 10-kDa fragment starting at ^{112}Q and ending probably on the carboxyl-terminal side of ^{193}E [see Figure 4 of Breton *et al.* (1990)] corresponds to a segment of about 80 residues starting at ^{137}L of *E. coli* GlnRS and located in its acceptor-binding domain near the ATP-binding site (Breton *et al.*, 1990; Rould *et al.*, 1989). ^{193}E is the first site for the V8 protease downstream of the GluRS acceptor-binding domain which, by comparison with the *E. coli* GlnRS, should end at the highly conserved ^{179}P. The short segment ^{125}C–^{138}C of *E. coli* GluRS contains most of the cysteines and histidines present in the 10-kDa zinc-binding fragment (Figure 9; Breton *et al.*, 1990) and is therefore likely to be involved in zinc coordination. It includes the ^{127}HSHEHH^{132} sequence, which contains the HXXEH motif present in the putative active site of an *E. coli* metalloendopeptidase, protease III (Becker *et al.*, 1992). Two histidines separated by three residues in an α-helix of a number of naturally occurring proteins are correctly positioned to chelate a metal ion (Higaki *et al.*, 1992). This ^{127}HSHEHH^{132} segment of *E. coli* GluRS is aligned with the ^{149}RSVEEN^{154} segments of *E. coli* GlnRS (Breton *et al.*, 1990), which is the beginning of the F helix (Rould *et al.*, 1989). This comparison suggests that ^{127}H and ^{131}H of *E. coli* GluRS have a good relative orientation to bind zinc. The tRNA acceptor helix binding domain of *E. coli* MetRS was also shown to contain the zinc-binding site of this class I aaRS (Landro & Schimmel, 1993).

Considering that zinc removal from GluRS, under conditions where the enzyme stays soluble, is accompanied by the loss of its aminoacylation activity (Figure 1), a decrease in its α-helix content (Figure 4), and a decreased mobility during electrophoresis on a 10% polyacrylamide gel under nondenaturing conditions (Figure 3), we conclude that zinc removal triggers a conformational change in GluRS to a more open structure which cannot catalyze the aminoacylation reaction. The location of the zinc atom in the tRNA acceptor helix binding domain of GluRS and the fact that the zinc-depleted conformer still binds $tRNA^{Glu}$ suggest that the (secondary) structure stabilized by this metal ion may be involved in the proper positioning of the 3′-end of $tRNA^{Glu}$ in the active site.

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