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# *Escherichia coli* Tyrosyl- and Methionyl-tRNA Synthetases Display Sequence Similarity at the Binding Site for the 3′-End of tRNA†

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ABSTRACT: Covalent modification of *Escherichia coli* tyrosyl-tRNA synthetase (TyrRS) by the 2′,3′-dialdehyde derivative of $tRNA^{Tyr}$ ($tRNA_{ox}$) resulted in a time-dependent inactivation of both ATP–$PP_i$ exchange and tRNA aminoacylation activities of the enzyme. In parallel with the inactivation, covalent incorporation of approximately 1 mol of [$^{14}$C]$tRNA_{ox}^{Tyr}$/mol of the dimeric synthetase occurred. Intact $tRNA^{Tyr}$ protected the enzyme against inactivation by the tRNA dialdehyde. Treatment of the TyrRS–[$^{14}$C]$tRNA^{Tyr}$ covalent complex with α-chymotrypsin produced two labeled peptides (A and B) that were isolated and identified by sequence analysis. Peptides A and B are adjacent and together span residues 227–244 in the primary structure of the enzyme. The three lysine residues in this sequence (lysines-229, -234, and -237) are labeled in a mutually exclusive fashion, with lysine-234 being the most reactive. By analogy with the known three-dimensional structure of the homologous tyrosyl-tRNA synthetase from *Bacillus stearothermophilus*, these lysines should be part of the C-terminal domain which is presumed to bind the cognate tRNA. Interestingly, the labeled TyrRS structure showed significant similarities to the structure around the lysine residue of *E. coli* methionyl-tRNA synthetase which is the most reactive toward $tRNA_f^{Met}$(ox) (lysine-335) [Hountondji, C., Blanquet, S., & Lederer, F. (1985) *Biochemistry 24*, 1175-1180].

Bacterial tyrosyl-tRNA synthetase (TyrRS)[1] is the object of many studies aimed at probing structure–activity relationships. The crystallographic structure of the *Bacillus stearothermophilus* enzyme is presently solved at 3-Å resolution (Bhat et al., 1982). It indicates two domains, one of which, the C-terminal domain, is thought to carry major determinants in tRNA binding. tRNA contacts with tyrosyl-tRNA synthetase have been studied by differential labeling of the lysine residues involved in complex formation (Bosshard et al., 1978). These contact regions include three lysines situated in the C-terminal half of the polypeptide chain of the enzyme (Winter et al., 1983). More recently, the portion of the cloned gene corresponding to the C-terminal region of the enzyme was deleted. The truncated tyrosyl-tRNA synthetase,

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[1] Abbreviations: TyrRS, tyrosyl-tRNA synthetase; MetRS, methionyl-tRNA synthetase; IleRS, isoleucyl-tRNA synthetase; TrpRS, tryptophanyl-tRNA synthetase; $tRNA_{ox}$ or tRNA dialdehyde, the dialdehyde formed by periodate oxidation of the 3′-ribose of tRNA; $tRNA_{ox-red}$, $tRNA_{ox}$ reduced with sodium borohydride; $adenosine_{ox-red}$, the adenosine derivative released from the 3′-end of $tRNA_{ox-red}$ by ribonuclease A; EDTA, ethylenediaminetetraacetic acid.

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lacking 100 C-terminal amino acid residues, catalyzes the formation of tyrosyl–adenylate but no longer binds $tRNA^{Tyr}$ or transfers tyrosine to $tRNA^{Tyr}$ (Waye et al., 1983).

The present work is carried out with the homologous tyrosyl-tRNA synthetase from *Escherichia coli* (Winter et al., 1983). It makes use of the fact that the lysine residues of a synthetase at the CCA binding site of tRNA can be covalently labeled by using periodate-oxidized tRNA (Fayat et al., 1979; Hountondji et al., 1979, 1980, 1985). Identification of the labeled peptide of tyrosyl-tRNA synthetase demonstrates that the C-terminal domain of the protein interacts with the CCA arm of tRNA. Moreover, comparison of the tyrosyl-tRNA synthetase primary structure at the binding site for the 3′-end of $tRNA^{Tyr}$ to the methionyl-tRNA synthetase corresponding structure reveals a specific sequence similarity.

## Materials and Methods

### *Materials*

Homogeneous tyrosyl-tRNA synthetase from *E. coli* strain EM20031 was purified as described (Fromant et al., 1981) and stored at −20 °C in 20 mM imidazole hydrochloride (pH 7.5), 10 mM 2-mercaptoethanol, 0.1 mM EDTA, and 50% glycerol. Enzyme concentration was determined from its absorbance at 280 nm by using a specific extinction coefficient of 1 unit·$mg^{-1}$·$cm^2$ and a molecular ratio of 95K, as deduced from the DNA sequence studies (Barker et al., 1982a). Tyrosine-specific tRNA (1300 pmol of tyrosine acceptance/$A_{260}$ unit of tRNA) was purchased from Boehringer (Mannheim). tRNA concentration was calculated from its extinction coefficient (24 $cm^2$·$mg^{-1}$) and molecular ratio (27 400) (Doctor et al., 1969). Ribonuclease A (60 Kunitz units/mg), chymotrypsin, and phenylmethanesulfonyl fluoride were from Sigma.

### *Methods*

*Inactivation Kinetics.* Periodate oxidation of $tRNA^{Tyr}$ was performed as described (Fayat et al., 1979). Inactivation of the enzyme (1 μM) was carried out in the presence of oxidized $tRNA^{Tyr}$ (10 μM) at 37 °C in 90 μL of 20 mM imidazole hydrochloride buffer (pH 8.0) containing 10 mM $MgCl_2$, 24% glycerol, and 2 mM sodium cyanoborohydride. At different times, 10-μL portions of the reaction mixture were withdrawn and quenched by 100-fold dilution in imidazole hydrochloride buffer (pH 7.5) containing 10 mM 2-mercaptoethanol and 200 μg/mL bovine serum albumin. The diluted portions were assayed for the amino acid dependent isotopic ATP–$PP_i$ exchange (Blanquet et al., 1974) and tRNA aminoacylation (Lawrence et al., 1973) activities.

[$^{14}$C]$tRNA^{Tyr}$ (specific radioactivity 15 000 counts·$min^{-1}$·$nmol^{-1}$) was prepared and oxidized as described (Fayat et al., 1979). Inactivation and labeling of TyrRS (15 μM) with [$^{14}$C]$tRNA_{ox}^{Tyr}$ (23 μM) were carried out as above in 360 μL. At various times, 5-μL aliquots were withdrawn, diluted, and assayed for enzymatic activity. Simultaneously, 25-μL aliquots were submitted to ribonuclease A digestion and trichloroacetic acid precipitation, as already described (Fayat et al., 1979).

*Chymotryptic Cleavage of the TyrRS–$tRNA_{ox}^{Tyr}$ Complex.* The TyrRS–[$^{14}$C]$tRNA_{ox}^{Tyr}$ covalent complex was obtained upon reacting 12 μM [$^{14}$C]$tRNA_{ox}^{Tyr}$ with 12 μM TyrRS, under the conditions given above. After 200 min, 73% of the initial enzymatic activity was lost, while 70% of the $^{14}$C label remained trichloroacetic acid insoluble after ribonuclease digestion. The reaction was then stopped by the addition of 25 mM $NaBH_4$ and the incubation mixture dialyzed overnight against 0.1 M $NH_4HCO_3$ (pH 8.2). The protein was treated with α-chymotrypsin for 240 min at 37 °C, at a protease to synthetase ratio of approximately 1/25 (w/w). The reaction was stopped by adding phenylmethanesulfonyl fluoride (0.5 mM final concentration). The chymotryptic digestion did not cause any degradation of the tRNA moiety, as verified by trichloroacetic acid precipitation of a [$^{14}$C]tRNA sample treated in parallel.

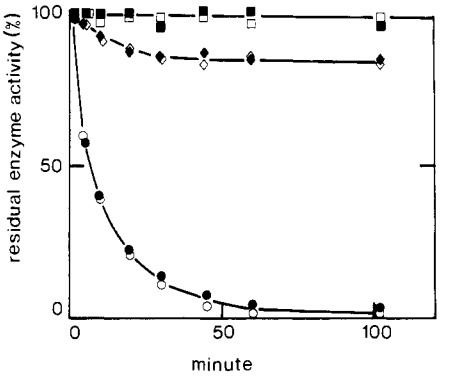


FIGURE 1: Time course of the inactivation of tyrosyl-tRNA synthetase by oxidized $tRNA^{Tyr}$. Tyrosyl-tRNA synthetase (1 μM) was incubated with 10 μM $tRNA_{ox}^{Tyr}$, as described under Methods. The residual aminoacylation (●, ■, ♦) and isotopic ATP–$PP_i$ exchange (○, □, ◇) activities were measured as a function of time. Control experiments were performed in the presence of 10 μM intact $tRNA^{Tyr}$ (■, □). For the protection experiments (♦, ◇), 60 μM of intact $tRNA^{Tyr}$ was present with the enzyme prior to $tRNA_{ox}$ addition.

*Peptide Mapping.* The two-dimensional thin-layer fingerprinting was performed with a CAMAG apparatus (Muttenz, Switzerland) according to Chen (1976), as described in Hountondji et al. (1985).

*Sequence Analysis.* Samples for amino acid analysis were hydrolyzed at 150 °C for 1 h in evacuated sealed tubes with 30 μL of trifluoroacetic acid and 60 μL of 5.7 N twice distilled HCl (Tsugita & Scheffler, 1982). Amino acid compositions were determined with an LKB 4400 amino acid analyzer. The results are expressed in molar ratios. Automatic degradation was carried out in a Beckman 890C sequenator with 0.1 M quadrol in the presence of polybrene. Phenylthiohydantoins were identified by high-pressure liquid chromatography (Lederer et al., 1983).

## Results

Both ATP–$PP_i$ exchange and aminoacylation activities of tyrosyl-tRNA synthetase were completely abolished upon prolonged incubation in the presence of excess oxidized $tRNA^{Tyr}$ (Figure 1). Intact $tRNA^{Tyr}$ protected the enzyme against inactivation by $tRNA_{ox}^{Tyr}$ (Figure 1). Covalent incorporation of [$^{14}$C]$tRNA_{ox}^{Tyr}$ followed enzyme activity loss (Figure 2). A total of 0.8 mol of oxidized [$^{14}$C]$tRNA^{Tyr}$ was incorporated per mole of dimeric tyrosyl-tRNA synthetase upon complete inactivation (Figure 2). This result suggests that the total inactivation of the dimeric enzyme activity can be reached upon the covalent attachment of only one $tRNA_{ox}$ molecule. Moreover, this conclusion is supported by the observation that, upon prolonged incubation of equimolar amounts of enzyme and $tRNA_{ox}$, the activity is destroyed by more than 50%. This is illustrated under Methods where, upon reacting 12 μM each of TyrRS and [$^{14}$C]$tRNA_{ox}^{Tyr}$, 73% of the initial enzyme activity was lost after 200 min.

Peptide purification from the labeled complex was carried out according to Hountondji et al. (1985). The strategy consisted of two steps. First, molecular sieving after α-chymotryptic digestion of the complex separated tRNA-labeled peptides from the bulk of the smaller unlabeled ones. Second,

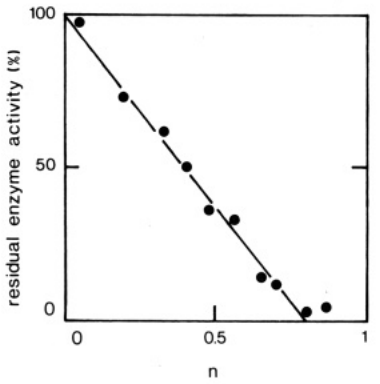


FIGURE 2: Labeling of tyrosyl-tRNA synthetase (15 $\mu$M) by [$^{14}$C]tRNA$^{Tyr}_{ox}$ (23 $\mu$M). The number of [$^{14}$C]tRNA$^{Tyr}_{ox}$ molecules ($n$) incorporated per mole of enzyme is represented as a function of the residual enzyme activity. The control experiment in the presence of intact [$^{14}$C]tRNA$^{Tyr}$ showed no incorporation of radioactivity.

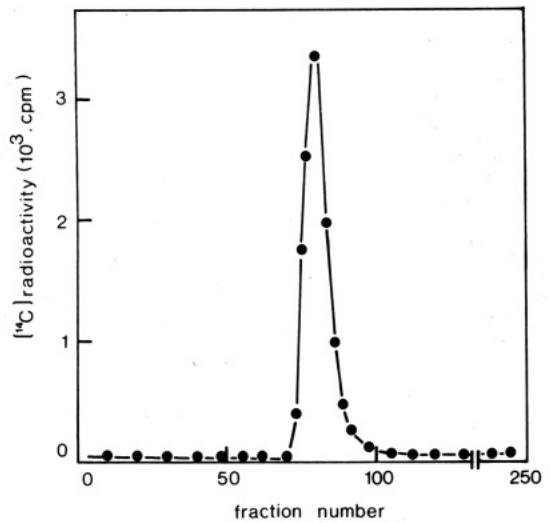


FIGURE 3: Chromatography on Sephadex G-50 of the chymotryptic digest of the TyrRS–[$^{14}$C]tRNA$^{Tyr}_{ox}$ complex. The peptide mixture prepared from 140 nmol of enzyme as described under Methods was loaded onto a Sephadex G-50 superfine column (68 × 1.2 cm) which was equilibrated and developed with 0.1 M ammonium acetate (pH 8.2) at a flow rate of 7 mL/h. Fractions of 0.4 mL were collected.

molecular sieving was repeated after ribonuclease treatment of the tRNA-labeled peptides, in order to separate the [$^{14}$C]adenosine-labeled peptides from the larger unlabeled ones. In the present study, the unreacted species, [$^{14}$C]tRNA$^{Tyr}_{ox-red}$ and enzyme, were not separated from the covalent complex prior to the chymotryptic digestion. Therefore, both the unreacted tRNA$_{ox-red}$ and the peptides covalently linked to tRNA eluted together in the void volume of the first Sephadex G-50 column (Figure 3). The radioactive fractions were pooled and further digested with ribonuclease A prior to a second chromatography on Sephadex G-50 (Figure 4). Two pools (I and II) were recovered that represented 67% and 22% of the radioactivity applied on the column, respectively. The two-dimensional peptide map of pool I (Figure 5a) showed two radioactive and fluorescamine-positive spots (A and B). A third spot (C) corresponded to the only spot observed on the peptide map of pool II (Figure 5b). This spot could not be stained with fluorescamine. Its amino acid composition as well as that of pool II before fingerprinting showed only glycine and trace amounts of a few other amino acids (not shown). Since glycine had been shown to arise from acid hydrolysis of ATP$_{ox-red}$ (Hountondji et al., 1985), we assumed that pool II represented the [$^{14}$C]adenosine$_{ox-red}$ released from the unreacted [$^{14}$C]tRNA$^{Tyr}_{ox-red}$ by the ribonuclease treatment. Spots

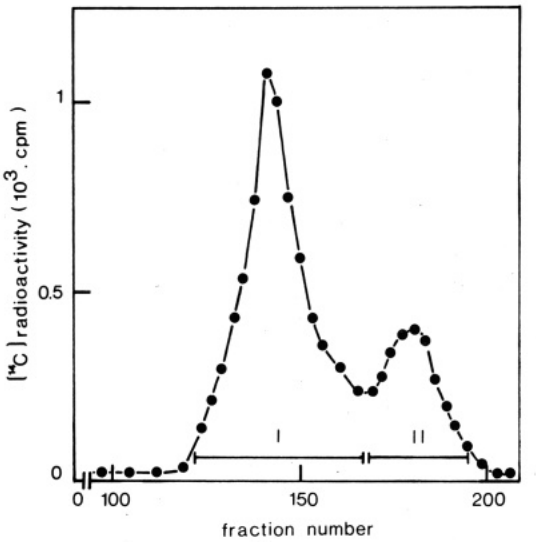


FIGURE 4: Fractionation of the $^{14}$C-labeled chymotryptic peptides. The radioactive material from Figure 3 was treated with pancreatic ribonuclease (200 $\mu$g in 0.1 M $NH_4HCO_3$, pH 8.2, 1 h at 37 °C), lyophilized, dissolved in 50 mM ammonium acetate (pH 8.2), and chromatographed on a column of Sephadex G-50 superfine (55 × 1.2 cm) which was equilibrated and run with the same buffer. The flow rate was 6.5 mL/h, and fractions of 0.3 mL were collected.

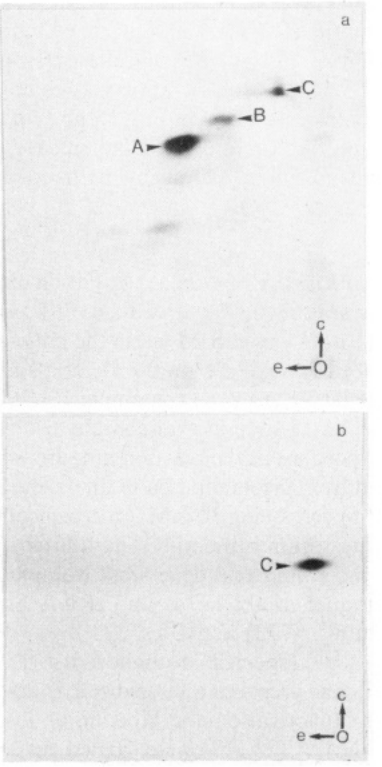


FIGURE 5: Autoradiograms of the two-dimensional peptide maps of pools I and II from Figure 4. The total $^{14}$C radioactivity spotted onto the thin-layer plate was 15 × $10^4$ and 4 × $10^4$ cpm for pools I and II, respectively (exposure 15 h with Kodak X-Omat X-ray film). Fluorescamine staining, performed as in Hountondji et al. (1985), did not show nonradioactive peptides. O, origin; e, electrophoresis; c, chromatography. (a) Pool I; (b) pool II.

A and B represented 41% and 7%, respectively, of the total radioactivity applied on the plate and all together 85% of the eluted radioactivity. None of the other weak spots was in sufficient amount to allow peptide identification.

The amino acid compositions of peptides A and B (Table I) corresponded to positions 227–235 (Ile-Thr-Lys-Ala-Asp-Gly-Thr-Lys-Phe) and 236–244 (Gly-Lys-Thr-Glu-Gly-Gly-

Table I: Amino Acid Compositions of Purified Chymotryptic Peptides Carrying $^{14}C$ Label[a]

| amino acid | A | B |
|---|---|---|
| Asp | 1.2 (1) | 0.4 (0) |
| Thr | 1.7 (2) | 0.9 (1) |
| Ser | 0.3 (0) | 0.4 (0) |
| Glu | 0.6 (0) | 1.6 (1) |
| Pro | | |
| Gly | 1.9 (1) | 2.8 (3) |
| Ala | 1.2 (1) | 1.2 (1) |
| Cys | | |
| Val | | *1* (1) |
| Met | | |
| Ile | 0.9 (1) | |
| Leu | | |
| Tyr | | |
| Phe | *1* (1) | |
| His | | |
| Lys | 1.0 (2) | (1) |
| Arg | | |
| amt (nmol) of peptide estd by | | |
| radioactivity | 0.92 | 0.76 |
| amino acid analysis | 0.93 | 0.74 |

[a] Peptide amounts were calculated from both radioactivity measurements and amino acid analysis. For peptides A and B, the amounts of each amino acid are normalized to Phe = 1 mol/mol and Val = 1 mol/mol, respectively. The figures in parentheses are the theoretical number of residues present in the peptides as deduced from the known sequence of *E. coli* TyrRS (Barker et al., 1982a). Values for contaminants equal to or less than 0.2 mol/mol are omitted.

Table II: Automated Edman Degradation of Peptide A[a]

Ile$^{227}$-Thr-Lys*-Ala-Asp-Gly-Thr-Lys*(Phe)$^{235}$

| cycle no. | residue no. in TyrRS sequence | amino acid | yield (nmol) | radioactivity (cpm) |
|---|---|---|---|---|
| 1 | 227 | Ile | 0.98 | 150 |
| 2 | 228 | Thr | 0.71 | 230 |
| 3 | 229 | Lys | 0.43 | 1080 |
| 4 | 230 | Ala | 0.45 | 380 |
| 5 | 231 | Asp | 0.56 | 150 |
| 6 | 232 | Gly | 0.66 | 150 |
| 7 | 233 | Thr | 0.39 | 150 |
| 8 | 234 | | | 5400 |
| 9 | 235 | | | 680 |

[a] Two nanomoles of peptide A (30 000 cpm of $^{14}C$ radioactivity) was subjected to automated Edman degradation in a spinning cup sequenator (Beckman 890C). Phenylthiohydantoins were identified by high-pressure liquid chromatography. The sequence thus established is shown, with solid arrows indicating residues positively identified as PTH-amino acids, the broken arrow indicating the residue that was not identified, and asterisks indicating residues for which radioactivity was found associated with the PTH fraction.

Ala-Val-Trp) in the sequence of the *E. coli* tyrosyl-tRNA synthetase (Barker et al., 1982a). Lysine was absent from peptide B analysis (Table I), indicating that the unique lysine residue of this peptide (lysine-237) was fully labeled. The presence of a lysine peak in peptide A analysis (Table I) suggested that both lysine residues in this peptide could not be fully labeled. To further identify the labeled lysine, peptide A was subjected to automated Edman degradation. Positive identification of all the residues was obtained with the exception of cycle 8 (Table II), where no phenylthiohydantoin (PTH) derivative was recovered. Instead, a new peak appeared close to PTH-Val, as already described (Hountondji et al., 1985), that contained a large part of the $^{14}C$ label. This demonstrated the major labeling of the lysine residue at this cycle (lysine-234). Table II shows that a small, but detectable, burst of $^{14}C$ radioactivity was observed at cycle 3. However, the positive identification of PTH-Lys and the small size of the new PTH peak showed that the lysine at this cycle (lysine-229) was slightly labeled.

## Discussion

Affinity labeling of *E. coli* tyrosyl-tRNA synthetase by oxidized $tRNA^{Tyr}$ yielded a specific covalent complex. Both the ATP–$PP_i$ exchange and tRNA aminoacylation activities are destroyed by the labeling. The stoichiometry of enzyme to tRNA incorporation is close to 1:1 in agreement with the fact that at equilibrium one molecule of dimeric enzyme binds one molecule of $tRNA^{Tyr}$ (Dessen et al., 1982).

Upon ribonuclease treatment of the 1:1 covalent complex, less than 5% of the initial ATP–$PP_i$ exchange activity of the enzyme is recovered (not shown). Since, after this treatment, each TyrRS dimer is supposed to contain only one covalently linked [$^{14}C$]adenosine moiety, it can be suggested that the covalent modification of one subunit also blocks the activity of the other unreacted subunit composing the dimeric synthetase. This behavior can be related to the catalytic coupling between *E. coli* and *B. stearothermophilus* TyrRS subunits, as documented in Jakes & Fersht (1975) and Fersht et al. (1975). The lack of activity of the ribonuclease-treated complex also argues for the strength of the subunit association under our experimental conditions. Otherwise, through reversible dissociation, a significant burst of activity would have been observed, corresponding to the statistical re-formation of fully active dimer molecules.

The chymotryptic digestion of the labeled enzyme yields two peptides (A and B) whose lysines have reacted with the 3′-end of the tRNA dialdehyde. These peptides are adjacent in the primary structure of *E. coli* tyrosyl-tRNA synthetase. In peptide B lysine-237 was fully labeled. In contrast, the Edman degradation experiment reported in Table II indicated clearly lysine-229 to have been only partially labeled. When an average repetitive yield of 90–95% in the Edman degradation is taken into account, it can be calculated that the radioactivity on lysine-229 represented 10–15% of the total counts present in peptide A. It is actually most probable that peptide A consisted of a mixture of two peptides with identical mobility, modified either at lysine-229 or at lysine-234. In the Edman degradation lysine-234 appeared to be fully modified, but the presence of 10–15% unmodified lysine would have been difficult to detect at cycle 8. In conclusion, the results show that lysines-229, -234, and -237 were labeled in a mutually exclusive fashion, with lysine-234 being the most reactive. This observation suggests at the same time a spatial clustering of these lysines.

Lysines-229, -234, and -237 belong to a sequence (Phe-220–Glu-239) that is highly conserved in the *B. stearothermophilus* tyrosyl-tRNA synthetase (Phe-216–Glu-235) (Winter et al., 1983). It is remarkable that the corresponding lysines of the *B. stearothermophilus* enzyme (Lys-225, Lys-230, and Lys-233) were slightly shielded from acetylation upon binding of the cognate tRNA, as shown by Bosshard et al. (1978). These authors could not determine, however, which part of the tRNA molecule was involved in the shielding process.

The structure of TyrRS from *B. stearothermophilus* consists of two domains: an N-terminal $\alpha/\beta$ domain that forms a "mononucleotide-binding fold" responsible for tyrosyl–adenylate binding and a C-terminal $\alpha$-helical domain thought to bind tRNA (Bhat et al., 1982; Waye et al., 1983). By analogy with this structural organization, the labeled lysines of the *E. coli* enzyme should be part of the C-terminal domain, in the

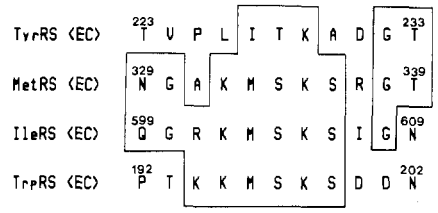


FIGURE 6: Alignment of the active site sequences of *E. coli* tyrosyl- and methionyl-tRNA synthetases and of similar sequences of IleRS and TrpRS. Identical and chemically similar residues (I = M, N = Q, R = K, and S = T) are boxed. The numbering indicates the distance from the N-terminus. The major labeled chymotryptic peptide from *E. coli* MetRS was Ser-334–Phe-340 (Hountondji et al., 1985). Sequence information for IleRS is taken from Webster et al. (1984), that for MetRS from Barker et al. (1982b) and Dardel et al. (1984), and that for TrpRS from Hall et al. (1982).

middle of the polypeptide joining strand F of the $\alpha/\beta$ domain to the first helix of the helical domain (Bhat et al., 1982). Since tyrosyl–adenylate makes contact with the continuation of the F strand toward the carboxyl terminus, this region of the enzyme is a reasonable target for the 3′-adenosine of $tRNA^{Tyr}$.

On the other hand, the reaction of the three clustered lysines suggests a certain mobility of the CCA arm of the complexed tRNA, or a dynamic disorder of this enzyme region, as indicated by the crystallographic studies (Bhat et al., 1982).

In a recent study, the lysines of *E. coli* methionyl-tRNA synthetase at the binding site of the 3′-end of $tRNA_f^{Met}$ have been identified (Hountondji et al., 1985). The predominantly labeled residue (Lys-335) belongs unambiguously to the C-terminal domain (Zelwer et al., 1982).

Lysine-335 in MetRS is clustered with two other lysines (Lys-332 and Lys-342). By comparison with TyrRS, these lysines might be capable of slightly reacting with $tRNA_f^{Met}$(ox). Following chymotryptic digestion of the MetRS–[$^{14}$C]-$tRNA_f^{Met}$ covalent complex, several spots could be observed on the thin-layer plates in addition to the identified major radioactive spots. These spots were too weak to be identified. However, two of them might correspond to fragments carrying labeled lysine-332 and lysine-342 fragments.

Lysine-335 of MetRS has not yet been definitely located in the 3-D structure of the synthetase. Recent realignment of the sequence on the model gives hope that lysine-335 could be located at the end of the fifth $\beta$-strand of the nucleotide binding fold, in a loop which is a flank of the active site, facing lysine-61 which is the other flank of the catalytic cavity (S. Brunie, personal communication). If it is confirmed by the structure refinement, such a location would fit nicely with that of the labeled lysines of TyrRS, at the C-terminus of the last $\beta$-strand composing the nucleotide binding domain (Blow et al., 1983).

The comparison between TyrRS and MetRS is reinforced by the observation of a clear similarity between the labeled amino acid sequences:


The similarity includes 5 identical residues (solid vertical lines) and 3 chemically similar ones (dotted lines), out of 12 compared residues. The possible functional significance of these amino acid sequences is further indicated by the occurrence in the available primary structures of IleRS (Webster et al., 1984) and TrpRS (Hall et al., 1982) of short amino acid stretches (Figure 6) strongly resembling the lysine-335 region of MetRS. The resemblance with TyrRS is poorer although significant, with a constant lysine flanked upstream by two chemically similar residues.

Sequence similarities between aminoacyl-tRNA synthetases have already been reported. In particular, a few synthetases share in their $NH_2$-terminal halves a short similar amino acid sequence including one cysteine and two histidine residues (Barker & Winter, 1982; Winter et al., 1983). In the cases of MetRS and TyrRS, this sequence similarity is emphasized by the fact that the cysteine and histidine residues occupy identical positions in the crystallographic structures of both enzymes (Barker & Winter, 1982; Blow et al., 1983). The present work, based on affinity labeling, introduces a new sequence similarity within the family of aminoacyl-tRNA synthetases. Upon chemical modification of the lysine residues in the sequences, activities of TyrRS and MetRS are lost. This suggests a functional role for those sequences.

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**Registry No.** Tyrosyl-tRNA synthetase, 9023-45-4; methionyl-tRNA synthetase, 9033-22-1.

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# Large-Scale Overproduction and Rapid Purification of the *Escherichia coli ssb* Gene Product. Expression of the *ssb* Gene under λ $P_L$ Control†

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ABSTRACT: We report a rapid procedure for the large-scale purification of the *Escherichia coli* encoded single-strand binding (SSB) protein, a helix-destabilizing protein which is essential for replication, recombination, and repair processes in *E. coli*. To facilitate the isolation of large quantities of the *ssb* gene product, we have subcloned the *ssb* gene into a temperature-inducible expression vector, $pPL_c28$ [Remaut, E., Stanssens, P., & Fiers, W. (1981) *Gene 15*, 81-93], carrying the bacteriophage λ $P_L$ promoter. A large overproduction of the *ssb* gene product results upon shifting the temperature of *E. coli* strains which carry the plasmid and also produce the thermolabile λcI857 repressor. After 5 h of induction, the *ssb* gene product represents ~10% of the total cell protein. The overexpression of the *ssb* gene and the purification protocol reported here enable one to isolate SSB protein (>99% pure) with final yields of ~3 mg of SSB protein/g of cell paste. In fact, very pure (>99%) SSB protein can be obtained after approximately 8 h, starting from frozen cells in the absence of any columns, although inclusion of a single-stranded DNA–cellulose column is generally recommended to ensure that the purified SSB protein possesses DNA binding activity. The ability to easily purify 1 g of SSB protein from 300–350 g of induced cells will facilitate physical studies requiring large quantities of this important protein.

The *Escherichia coli ssb* gene product is a necessary component in replication, recombination, and repair processes of that bacterium (Wickner & Hurwitz, 1974; Meyer et al., 1979; Johnson, 1979; McEntee et al., 1980; Glassberg et al., 1979). It is a member of a class of nucleic acid binding proteins referred to as "helix-destabilizing proteins" (Alberts & Sternglanz, 1978), which bind selectively and in most cases cooperatively to single-stranded conformations of nucleic acids (Sigal et al., 1972; Kowalczykowski et al., 1981; Lohman et al., 1986).

Until recently, physical studies of the *E. coli* single-strand binding (SSB) protein and its interactions with nucleic acids have been limited due to the difficulty of routinely obtaining multimilligram quantities of the purified protein. The cloning of the *ssb* gene into multicopy plasmids (Sancar & Rupp, 1979; Chase et al., 1980; Sancar et al., 1981), resulting in increased levels of *ssb* gene expression, has made the purification of tens of milligrams of SSB protein a routine matter, although most protocols require two to three columns (Sigal et al., 1972; Chase et al., 1980). We have improved the purification procedure further by subcloning the *ssb* gene into a plasmid containing the strong bacteriophage λ $P_L$ promoter so that the *ssb* gene is under transcriptional control of the λ $P_L$ promoter. This procedure has been used to overexpress a number of prokaryotic proteins (Bernard et al., 1979; Remaut et al., 1981; Shimatake & Rosenberg, 1981; Yoakum et al., 1982; Gribskov & Burgess, 1983; Shigesada et al., 1984; Mott et al., 1985) including the *E. coli* SSB-1 protein, a temperature-sensitive mutant of the SSB protein (Williams et al., 1984), as well as some eukaryotic proteins (Rosenberg et al., 1983). Transcription of the gene from the λ $P_L$ promoter can be controlled by the growth temperature in *E. coli* strains producing a temperature-inducible λ cI repressor (e.g., cI857); at 30 °C, transcription is almost fully repressed, whereas at 42 °C, transcription is fully derepressed (Remaut et al., 1981). This leads to large increases in mRNA and protein levels, assuming that no major problems exist, as is the case with the *E. coli ssb* gene product.

We also report a very rapid procedure for the purification of the *ssb* gene product from *E. coli* harboring the inducible, overproducing plasmid. This procedure enables one to purify large quantities of the SSB protein (>99% pure) from small amounts of induced cells in a matter of several hours in the absence of any columns. However, a single-stranded DNA–cellulose column is recommended as part of the purification

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