JOURNAL OF BACTERIOLOGY, Sept. 1977, p. 943-950
Vol. 131, No. 3

Printed in U.S.A.

Threonyl-Transfer Ribonucleic Acid Synthetase from *Escherichia coli*: Subunit Structure and Genetic Analysis of the Structural Gene by Means of a Mutated Enzyme and of a Specialized Transducing Lambda Bacteriophage

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Received for publication 14 March 1977

Threonyl-transfer ribonucleic acid synthetase (ThrRS) has been purified from a strain of *Escherichia coli* that shows a ninefold overproduction of this enzyme. Determination of the molecular weight of the purified, native enzyme by gel chromatography and by polyacrylamide gel electrophoresis at different gel concentrations yielded apparent molecular weight values of 150,000 and 161,000, respectively. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate yields a single protein band of 76,000-dalton size. From these results an α_2 subunit structure can be inferred. A mutant with a structurally altered ThrRS, which had been obtained by selection for resistance against the antibiotic borrelidin, was used to map the position of the ThrRS structural gene (*thrS*) by P1 transductions. It was found that *thrS* is located in the immediate neighborhood of *pheS* and *pheT*, which are the structural genes for the α and β subunits of phenylalanyl-transfer ribonucleic acid (tRNA) synthetase, the gene order being *aroD-pheT-pheS-thrS*. A λ phage that was previously shown to specifically transduce *pheS*, *pheT*, and also the structural gene for the translation initiation factor IF3 can complement the defect of the altered ThrRS of the borrelidin-resistant strain. This phage also stimulates the synthesis of the 76,000, molecular-weight polypeptide of ThrRS in ultraviolet light-irradiated. *E. coli* cells. These results indicate that the genes for ThrRS, α and β subunits of phenylalanyl-tRNA synthetase, and initiation factor IF3 are immediately adjacent on the *E. coli* chromosome.

The antibiotic borrelidin inhibits specifically the threonyl-transfer ribonucleic acid synthetase (ThrRS) of procaryotic and eucaryotic cells (9, 25). Mutants of *Escherichia coli* that are resistant to borrelidin can be classified into two groups: strains belonging to the first group possess a structurally altered ThrRS, and those belonging to the second one have increased levels of the enzyme (19). The ThrRS of one of these overproducing strains has been purified, and, by the biochemical and immunological criteria used, it was shown that it is indistinguishable from the ThrRS of the parental strain, indicating that some mechanism involved in the regulation of the level of this enzyme is affected by the mutation (22).

The knowledge of the location of the structural gene(s) for the ThrRS is a basic prerequisite for investigating the regulatory site affected in these ThrRS-overproducing strains, since one of the first questions to be answered is whether or not the corresponding mutation is linked to the structural gene. Until now no information has been available about the precise location of the gene coding for the ThrRS polypeptide(s), and therefore it was the aim of this study to determine the chromosomal location of this gene (*thrS*) by means of a borrelidin-resistant mutant exhibiting a structurally altered ThrRS. In addition, this paper shows that the ThrRS most likely consists of only one kind of polypeptide chain and that its structural gene is carried by a specialized transducing phage λ, which also carries the genes for phenylalanyl-transfer ribonucleic acid (tRNA) synthetase and for the translation initiation factor IF3 (8, 27; M. Springer, M. Graffe, and H. Hennecke, Proc. Natl. Acad. Sci. U.S.A., in press).

MATERIALS AND METHODS

Organisms and media. The bacterial strains and the phages used in this work are listed in Table 1. The media and growth conditions were the same as

TABLE 1. *Bacterial strains and phages used*

Organism and strain	Characterization[a]	Source or derivation
E. coli		
K-12B	Wild type	Reference 2
K-12B Bor^r-8	*thrS8*, structurally altered ThrRS	Reference 19
K-12B Bor^r-15	Elevated ThrRS level	Reference 19
KL159	*thi-1 his-4 aroD5 proA2 recA1 xyl-5* or *xyl-7 nalA12 tsx-1?* or *tsx-29?* λ^- *supE44*	Acridine orange "curing" of KLF48/KL159
KLF44/KL251	F' *thi-1 metE70 trpE38 purE42 proC32 leu-6 recA1 mtl-1? xyl-5 ara-14 lacZ36 azi-6 str-109 tonA23 tsx-67* λ^- *supE44?* Episome F144	B. Bachmann
KLF48/KL159	Like KL159, with episome F148	B. Bachmann
AB1360	F$^-$ *thi-1 argE3 his-4 proA2 aroD6 lacY1 galK2 mtl-1 xyl-5 tsx-29 supE44?*	A. L. Taylor via B. Bachmann (24)
Bor811	F$^-$ *thrS8 thi-1 argE3 his-4 aroD6 proA2 lacY1 galK2 mtl-1 xyl-5 tsx-29 supE44?*	P1 transduction: K12B Bor^r-8 → MC104
HH36	F$^-$ *thrS8 pheT354 thi-1 argE3 his-4 proA2 lacY1 galK2 mtl-1 xyl-5 tsx-29 supE44?*	P1 transduction: MC1440 → Bor811
MC104	F$^-$ *pheS12 pheT354 thi-1 argE3 his-4 aroD6 proA2 lacY1 galK2 mtl-1 xyl-5 tsx-29 supE44?*	Reference 6
MC1440	F$^-$ *pheS12 pheT354 thi-1 argE3 his-4 proA2 lacY1 galK2 mtl-1 xyl-5 tsx-29 supE44?*	Reference 6
159(λ*ind*)	*rpsL sup*$^+$ *gal* UV^s (λ*ind*)$^+$	Reference 26: Springer et al., in press
S. parvulus Tü113	Produces borrelidin	K. Poralla
Phages		
λwt	Wild type	M. Gottesman
λp2	c1857, *sR*Iλ$_3$°, *sR*Iλ$_2$°, *SRI*λ$_1$°, (*int xis exo* β γ cIII)Δ *E. coli* DNA between the two *Eco*RI sites *s*RIλ$_4$ and *s*RI$_{lac}$.	References 7, 23; M. Gottesman; Kourilsky, Perricaudet, and Tiollais (manuscript in preparation)

[a] Gene symbols were adopted from Bachmann et al. (1).

described previously (6). Strain K-12B Bor^r-15, which was used as a source for the purification of ThrRS, was grown in 10 liters of rich medium to an absorbancy at 420 nm of 7.0 in a New Brunswick M19 fermentor. The cells were harvested by centrifugation, washed once in buffer A (20 mM potassium phosphate [pH 7.5]–10% glycerol–6 mM 2-mercaptoethanol–0.1 mM sodium ethylenediaminetetraacetate) (22) containing 10 mM magnesium acetate, and stored frozen at −20°C until used.

Purification of ThrRS. ThrRS was purified by following a modification of the procedure outlined previously (22). A 100-g (wet weight) amount of cells of strain K-12B Bor^r-15 was suspended in 200 ml of buffer A containing 10 mM magnesium acetate and 2 μg of deoxyribonuclease per ml and passed through a French press cell at 20,000 lb/in^2. The homogenate obtained was first centrifuged for 45 min at 20,000 × *g* (2°C) and then clarified by centrifugation for 210 min at 100,000 × *g* (2°C).

The 100,000 × *g* supernatant was subjected to ammonium sulfate fractionation by the addition of a saturated solution made up in buffer A (22).

Protein fractions precipitating at concentrations of 38, 50, and 55% were collected by centrifugation for 20 min at 8,000 × *g*, dissolved in a minimum of buffer A, and dialyzed overnight against 5 liters of buffer A. The protein concentration of these fractions and of the 100,000 × *g* supernatant was determined by the Folin phenol method (16), using bovine serum albumin as standard; 2.5- and 5-μg amounts were taken to assay the ThrRS activity, essentially as described by Paetz and Nass (22). As the ThrRS activity was equally distributed in the 50 and 55% fractions, they were pooled and applied together to a diethylaminoethyl-cellulose column (20 by 5 cm) that had been equilibrated in buffer A. The column was eluted with a linear gradient of potassium phosphate (20 mM to 0.4 M in buffer A; 1,000 ml/1,000 ml; 40 ml/h) (22).

Fractions with ThrRS activity were pooled and dialyzed against 20 mM potassium phosphate buffer, pH 6.8, containing 10% glycerol and 6 mM 2-mercaptoethanol. The enzyme solution was absorbed at 22 ml/h on an hydroxylapatite column (30 by 2.5 cm; Bio-Rad), which was equilibrated with the same buffer. The column was eluted with a linear gradient of potassium phosphate buffer, pH 6.8 (20 mM to 0.4 M; 500 ml/500 ml; 22 ml/h). Samples of the fractions showing ThrRS activity were subjected to polyacrylamide gel electrophoresis. At this stage of the purification procedure the enzyme was about 80 to 90% pure; it contained one major contaminating band migrating somewhat slower in the disk gel (see Fig.

1). To remove the impurities, the protein solution was further fractionated, first by Sephadex G200 gel filtration and then by hydroxylapatite chromatography at pH 7.5. For this purpose, the enzyme solution was concentrated by ultrafiltration, using a PM30 membrane filter (Aminco), and then loaded onto a Sephadex G200 column (90 by 2.5 cm). The column was equilibrated and eluted (5 ml/h) with 20 mM tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.5, containing 0.1 M KCl and 0.5 mM dithioerythritol. The ThrRS-containing fractions were pooled and absorbed on a second hydroxylapatite column (30 by 2.5 cm) equilibrated in 20 mM potassium phosphate, pH 7.5, containing 10% glycerol and 6 mM 2-mercaptoethanol. Elution was with a 2-liter linear gradient ranging from 20 mM to 0.3 M potassium phosphate. The enzyme was eluted from this column as a single symmetrical peak. Polyacrylamide gel electrophoresis showed that it did not contain any visible impurities (Fig. 1). The purification started out from 6,350 mg of ribosome-free supernatant protein with a specific ThrRS activity of 0.28 μmol of L-threonine attached to tRNA per mg of protein per h (at 37°C, pH 7.5; L-threonine concentration, 20 μM). A total of 13 mg of purified enzyme with a specific activity of 27.5 was obtained, the enrichment thus being 98-fold at a yield of 20%.

Samples of purified leucyl-tRNA synthetase and isoleucyl-tRNA synthetase were kindly donated by E. Holler. Phenylalanyl-tRNA synthetase was purified as described previously (12).

Isolation of borrelidin. A crude borrelidin fraction was isolated from the culture fluid of *Streptomyces parvulus* Tü113, essentially as described by Hütter et al. (9) with the exception that the final purification step (Craig distribution) was omitted. The crude antibiotic obtained was suitable for the discrimination between the borrelidin-resistant and -sensitive enzyme forms by in vitro and in vivo tests.

Enzyme assays. ThrRS activity was assayed as described previously (22). The activity of other aminoacyl-tRNA synthetases was determined by using the respective amino acid at a concentration of 20 μM and at a specific radioactivity of 10 μCi/μmol. The activities of fructose-1,6-diphosphate aldolase and L-lactate dehydrogenase were determined spectrophotometrically as described by Bergmeyer (3).

Measurement of the molecular weight of the native ThrRS. (i) Gel filtration. A 1-mg portion of purified ThrRS was loaded on a Sephadex G200 column (90 by 2.5 cm) together with 1 mg of purified phenylalanyl-tRNA synthetase and 5 mg each of fructose-1,6-diphosphate aldolase (rabbit muscle) and L-lactate dehydrogenase (rabbit muscle). The column was eluted with 20 mM tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.5–0.1 M KCl–0.5 mM dithioerythritol at a rate of 5 ml/h; 5-ml fractions were collected, and samples of them were assayed for enzyme activity.

(ii) Polyacrylamide gel electrophoresis at different gel densities. The determination of the electrophoretic mobilities of proteins in polyacrylamide gels of different concentrations allows the calculation of the approximate molecular weight, provided the reference proteins used possess the same partial specific volumes as the protein tested (for review, see Maurer [17]). To apply this method to the estimation of the molecular weight of ThrRS, 10 μg of the enzyme was subjected to electrophoresis together with about the same quantities of phenylalanyl-tRNA synthetase, isoleucyl-tRNA synthetase, and leucyl-tRNA synthetase at gel concentrations of 6, 7.5, and 9% acrylamide, respectively. The gels were run at 2 mA/tube (100- by 6-mm tubes) until the marker dye (bromophenol blue) had reached the bottom. They were stained with 1% amido black in 7% acetic acid and destained by diffusion in 7% acetic acid. The migration distance of the proteins was used to calculate the approximate molecular weight (17).

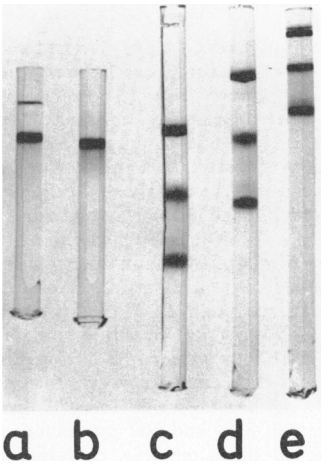

FIG. 1. *Polyacrylamide gels (7.5%) of (a) the purified ThrRS preparation after the first hydroxylapatite chromatography and (b) the eluate from the second hydroxylapatite column. (c), (d), and (e) show the migration of (from top) phenylalanyl-tRNA synthetase, ThrRS, and isoleucyl-tRNA synthetase in 6, 7.5, and 9% gels, respectively.*

Molecular weight determination of the ThrRS polypeptide by SDS-polyacrylamide gel electrophoresis. The molecular weight of the constituent polypeptide of ThrRS was determined by polyacrylamide slab gel electrophoresis in the presence of sodium dodecyl sulfate (SDS). The samples were denatured by heating in "sample" buffer (13) and then applied to the 5% stacking gel/10% separation gel system described by Laemmli (13). Electrophoresis was carried out in the apparatus devised by Studier (28). Gels were run with 10 mA, stained with 0.1% Coomassie brilliant blue in 7.5% acetic acid and 50% methanol, and destained by diffusion in 7.5% acetic acid and 50% methanol.

Transductions. Transductions, selection of *aroD*$^+$ recombinants, and testing of the *pheS* and *pheT* markers were carried out as previously described (6). Presence or absence of the borrelidin resistance allele was checked by assay of the ThrRS in crude cell-free extracts. The enzyme of the borrelidin-resistant strain can easily be distinguished from the

wild-type ThrRS by its considerably lower specific activity (19).

Infection of ultraviolet light-irradiated cells with λp2 and analysis of the proteins synthesized. The procedures for inactivating the recipient cells by irradiation with ultraviolet light and the electrophoretic separation and fluorographic detection of the proteins synthesized in λ-infected cells were essentially as described recently (8; Springer et al., in press), except that the radioactive labeling was done with 4 μCi of a *U*-^{14}C-labeled L-amino acid mixture per ml (New England Nuclear Corp., NEN445).

Preparation of the antiserum. Rabbits were immunized with the purified ThrRS preparation described above by subcutaneous injection on 3 consecutive days, each for a period of 3 weeks. The protein amounts injected on each day were: first week: 200, 100, and 100 μg; second week: 100, 200, and 300 μg; third week: 200, 300, and 500 μg. Before injection, the enzyme preparation was dialyzed overnight against 1 mM potassium phosphate buffer (pH 8.0) containing 0.9% sodium chloride. First-week injections were mixed in a 1:1 (vol/vol) ratio with the complete form of Freund adjuvant; second- and third-week injections were made up in the incomplete adjuvant. About 40 ml of blood was collected from the ear vein 5 days after the last antigen dose. The immunoglobulin fraction was isolated from it by following a procedure described recently (23).

RESULTS

Genetic mapping of the structural gene (*thrS*) for ThrRS by means of a structurally altered enzyme. For a first approximate location of *thrS*, use was made of the fact that the specific activity of aminoacyl-tRNA synthetases follows a distinct gene dosage effect (4, 5, 23). We have therefore determined the enzyme activity of extracts of different merodiploid strains with different episomes which cover most of the *E. coli* chromosome. It was found that only one merodiploid strain, KLF48/KL159, showed an increased ThrRS activity value (Table 2). KLF148 contains part of the *E. coli* chromosomal segment between the *his* and *aroD* markers and therefore also harbors the structural genes for phenylalanyl-tRNA synthetase (*pheS*, *pheT*), which explains the increased phenylalanyl-tRNA synthetase activity observed. The lack of response of arginyl-tRNA synthetase, which also maps in this chromosomal area, to gene dosage is possibly due to the fact that episome F148 carries a deletion (15) between *cheB-cheC*, which might include the loss of the *argS* gene.

Next, whether the *thrS* gene could be cotransduced with the *aroD* marker via phage P1*kc* was tested. For this purpose, the phage was grown on one of the borrelidin-resistant mutants with structurally altered ThrRS (strain K-12B Bor^r-8) (19) and used to transduce strain MC104, which is *aroD* and carries a temperature-sensitive mutation in *pheT* and a *p*-fluorophenylalanine resistance mutation in *pheS* (6). The phenotypes of the *aroD*$^+$ recombinants obtained are listed in Table 3. It is clear that *thrS* is very closely linked to the structural genes for the α and β subunits of phenylalanyl-tRNA synthetase, since they are separated by only a 5% crossover. Since none of the recombinants had inherited *thrS* without concomitantly acquiring the *pheS* and *pheT* alleles from the donor strain, since about 5% of the transductants had obtained *pheS* and *pheT* without *thrS*, and since those recombinants (3%) with crossovers between the *pheS* and *pheT* mutations had maintained the recipient *thrS* allele, it is clear that the gene order is *-aroD-pheT-pheS-thrS*.

Molecular weight of the native ThrRS. A few aminoacyl-tRNA synthetases from *E. coli* consist of two dissimilar polypeptide chains (for review, see reference 21). To decide whether ThrRS belongs to this class of synthetases and whether a second structural gene could exist, therefore, we attempted to determine the subunit structure of this enzyme. For this purpose ThrRS was purified from strain K-12B Bor^r-15, which shows a ninefold overproduction of this enzyme, by using the purification protocol outlined under Materials and Methods. From 100 g (wet weight) of cells about 13 mg of apparently homogeneous enzyme (Fig. 1) was obtained.

To estimate the molecular weight of the purified ThrRS, a sample of the enzyme was subjected to Sephadex G200 gel filtration together

TABLE 2. *Specific aminoacyl-tRNA synthetase activities of extracts from strains K-12B, KL159, KLF44/KL251, and KLF44/KL251*

Strain	Sp act (μmol/h per mg of protein)		
	ThrRS[a]	Phenylalanyl-tRNA synthetase	Arginyl-tRNA synthetase
K-12B	0.211	0.049	0.111
KLF48/KL159	0.366	0.098	0.113
KL159	0.222	ND[b]	0.121
KLF44/KL251	0.218	ND	0.119

[a] Concentration of L-[^{14}C]threonine in the assay was 0.4 mM.
[b] ND, Not determined.

TABLE 3. *Evidence for the gene order aroD-pheT-pheS-thrS*

aroD$^+$ transductants	No.	%
pheT$^+$ *pheS*$^+$ *thrS8*	65	67.7
pheT354 pheS12 thrS$^+$	23	24.0
pheT$^+$ *pheS12 thrS*$^+$	3	3.1
pheT$^+$ *pheS*$^+$ *thrS*$^+$	5	5.2

with three reference proteins of known molecular weights (Fig. 2). Figure 2 shows that the purified ThrRS is eluted from the column between fructose-1,6-diphosphate aldolase (molecular weight, 160,000) and L-lactate dehydrogenase (molecular weight, 140,000), the elution position corresponding to a molecular weight of approximately 150,000.

In a separate experiment, a crude extract of *E. coli* B was subjected to gel filtration, and the elution position of ThrRS was compared with those of other aminoacyl-tRNA synthetases of known size (not shown). Consistent with results obtained previously (18), the ThrRS eluted immediately after the methionyl-tRNA synthetase (molecular weight, 173,000; 7, 14).

As an independent method for the determination of the molecular weight of the native form of ThrRS, polyacrylamide gel electrophoresis in gels of different densities was used (17). From the electrophoretic mobility of ThrRS relative to those of purified phenylalanyl-tRNA synthetase and isoleucyl-tRNA synthetase in 6, 7.5, and 9% polyacrylamide gels (see Fig. 1), an average molecular weight of 164,000 could be calculated (17). In a parallel experiment, the electrophoretic mobility was compared with those of leucyl-tRNA synthetase and phenylalanyl-tRNA synthetase, and in this case a molecular weight of 157,000 was obtained; the average molecular weight of the native enzyme with the electrophoretic method, therefore, is approximately 161,000.

Molecular weight of ThrRS under denaturing conditions. To obtain information on the size of the constituent polypeptide chain of ThrRS, the enzyme was denatured in SDS-containing "sample" buffer (13) and subjected to polyacrylamide gel electrophoresis in the presence of SDS (see Fig. 5). Figure 3 shows a plot of the migration distance of ThrRS and several reference proteins versus the logarithm of the molecular weight. ThrRS in the denatured form is resolved as a single band in the SDS gel; the migration distance relative to the marker proteins used indicates its size to be 76,000 ± 3,000 daltons.

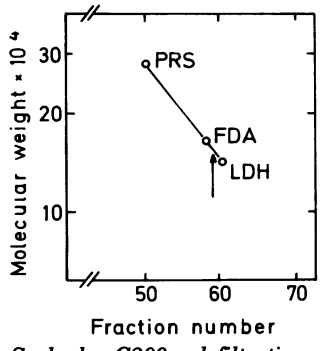

FIG. 2. *Sephadex G200 gel filtration of purified ThrRS. The arrow indicates the elution position of ThrRS in relation to those of purified phenylalanyl-tRNA synthetase from E. coli (PRS), fructose-1,6 diphosphate aldolase from rabbit muscle (FDA), and L-lactate dehydrogenase from rabbit muscle (LDH).*

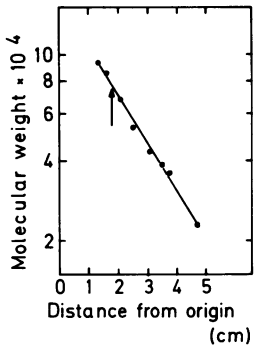

FIG. 3. *Molecular weight determination of ThrRS by polyacrylamide gel electrophoresis in the presence of SDS. The marker proteins (from top) are: phenylalanyl-tRNA synthetase, β subunit (molecular weight, 94,000); ovalbumin dimer (molecular weight, 86,000); bovine serum albumin (molecular weight, 68,000); glutamate dehydrogenase (molecular weight, 53,000); ovalbumin monomer (molecular weight, 43,000); phenylalanyl-tRNA synthetase, α subunit (molecular weight, 38,000); L-lactate dehydrogenase (molecular weight, 35,000); α-chymotrypsinogen (molecular weight, 23,000). The arrow indicates the position of the ThrRS protein band.*

Evidence that a specialized transducing λ phage isolated for transduction of the genes for phenylalanyl-tRNA synthetase also carries *thrS*. A λ phage (λp2) has been described recently which specifically transduces the phenylalanyl-tRNA synthetase structural genes *pheS* and *pheT* and the gene for initiation factor IF3 (8, 27; Springer et al., in press). Upon infection of ultraviolet light-irradiated cells, this phage also induces the synthesis of a polypeptide of 78,000-dalton size (8; Springer et al., in press). The close correspondence of the molecular weight of this protein and of the ThrRS subunit and the proximity of the *thrS* and the *pheS* and *pheT* genes prompted an investigation of whether the unknown protein coded by the phage is identical to the ThrRS polypeptide.

To this end, whether phage λp2 is able to complement the *thrS8* mutation was first studied. The *thrS8* lesion is responsible for the synthesis of a structurally altered ThrRS, which can no longer be inhibited by borrelidin in vivo and in vitro; it thereby can be easily distin-

guished from the borrelidin-sensitive, wild-type enzyme, the structural gene of which is possibly carried by phage λp2. A lysate of λp2 was used together with wild-type helper phages to infect strain HH36, which is *thrS8* (borrelidin resistance mutation) and *pheT354* (temperature-sensitive phenylalanyl-tRNA synthetase). A temperature-resistant transductant of strain HH36, namely, HH36λp2, was grown up, and crude extracts of it were tested for inhibition of the ThrRS activity by borrelidin in comparison to extracts from the uninfected parental strain HH36 and the *thrS*$^+$ strain AB1360. Figure 4 shows that the specific ThrRS activity of the λ lysogen HH36λp2 is higher than that of the *thrS*$^+$ strain AB1360, which could be the consequence of an increased gene dosage. More important, however, is the finding that the ThrRS activity of strain HH36, which is not affected by borrelidin, is replaced by a borrelidin-sensitive enzyme in the λ transductant. That borrelidin inhibits ThrRS activity even below the level of activity present in HH36 extracts can be explained on the basis that in the *thrS*$^+$/*thrS8* heteromerodiploid the two kinds of polypeptide chains (borrelidin-resistant and -sensitive ones) can associate statistically and only those aggregates that solely contain "borrelidin-resistant" subunits are borrelidin resistant. Taken together, these results strongly support the notion that λp2 indeed carries *thrS*.

Direct evidence for this was finally brought about by the analysis of the proteins synthesized in ultraviolet-irradiated cells after infection with λp2 (Fig. 5). Ultraviolet-light irradiation causes nearly total loss of the protein synthesis capacity of the recipient strain 159λ*ind* (Fig. 5B, lane 4). After infection with λp2 the cells are able to synthesize four proteins (I to IV), which are resolved in our gel system, and probably a fifth protein (Springer et al., in press), which migrates with the front (Fig. 5B, lanes 5 and 6). These λp2-coded proteins are under the control of *E. coli* promoters and genes, since the repressor of λ*ind* does not allow the expression of λ-specific genes (Springer et al., in press). Proteins I, III, and IV have recently been identified as the β and α subunits of phenylalanyl-tRNA synthetase (8) and as the ribosomal initiation factor IF3 (Springer et al., in press), respectively. There is strong evidence that protein II is the ThrRS subunit: (i) it has exactly the size of the purified ThrRS subunit (cf. Fig. 5A, lane 3, with Fig. 5B, lanes 5 and 6), and (ii) this protein cross-reacts with an antiserum directed against the purified ThrRS (Fig. 5B, lanes 7 and 8). The anti-ThrRS serum does not precipitate the β and α subunits of phenylalanyl-tRNA synthetase. The radioactive material migrating at the front of the electropherogram possibly consists of unspecifically precipitated material or of prematurely terminated polypeptides still recognized by the antiserum (see also reference 8).

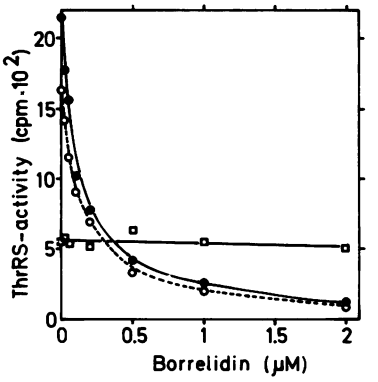

FIG. 4. *Inhibition of the ThrRS activity of crude extracts from strains AB1360* (○), *HH36* (□), *and HH36λp2* (●) *by borrelidin.*

DISCUSSION

With two independent methods, the molecular weight of the native ThrRS was determined to be between 150,000 and 161,000. The size of the constituent polypeptide chain was found to be 76,000 daltons. Thus, like methionyl-tRNA synthetase (11), ThrRS from *E. coli* seems to belong to that group of aminoacyl-tRNA synthetases possessing an α_2 subunit structure, where α has a molecular weight roughly between 75,000 and 90,000 (21). The identity of the two monomers in the oligomeric enzyme has still to be supported by protein-chemical criteria, although the existence of only one band in SDS gels and of one single spot in O'Farrell electropherograms (20) are in favor of it.

The existence of single-step mutants with structurally altered ThrRS (10, 19) suggests that in *E. coli* there is only one copy of the *thrS* cistron, which recently was reported to be located around 30 min on the chromosome (10). From the genetic data presented in this paper, the ThrRS structural gene is located precisely at 37.7 min on the *E. coli* chromosome map (1) in the immediate neighborhood of the phenylalanyl-tRNA synthetase structural genes. The close proximity of these genes is quite unique, since the structural genes for the other aminoacyl-tRNA synthetases so far known are scattered around the *E. coli* genome (1, 21).

The experimental results obtained with the specialized transducing phage λp2 agree well

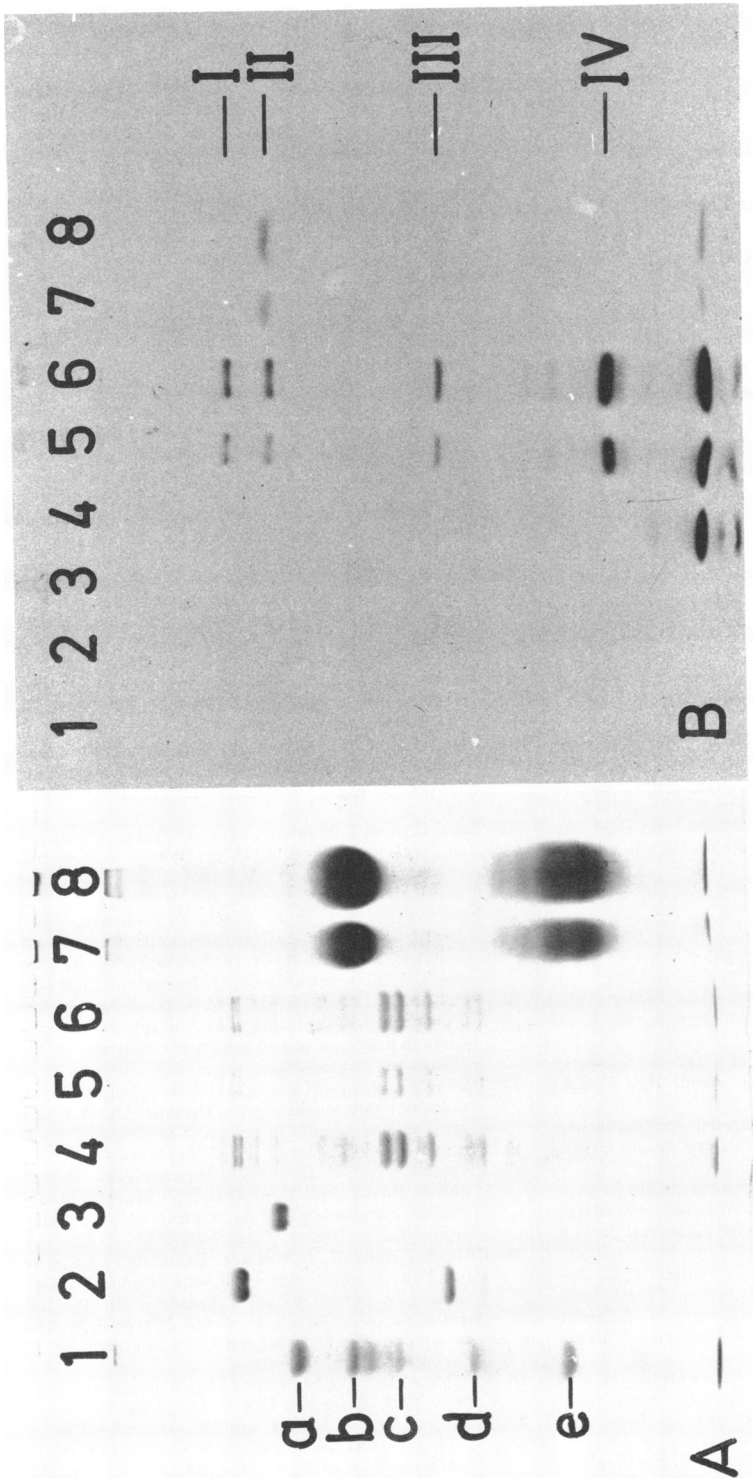

FIG. 5. *Scintillation autograph of the SDS-gel electropherograms of radioactively labeled crude extracts from E. coli strain 159λind infected with λp2. (A) Staining pattern of the wet gel; (B) autoradiogram of the same slab gel after drying. Lane 1 shows the migration position of the reference proteins (from top): (a) bovine serum albumin, (b) glutamate dehydrogenase, (c) ovalbumin monomer, (d) L-lactate dehydrogenase, and (e) chymotrypsinogen. Lanes 2 and 3 received samples of purified phenylalanyl-tRNA synthetase and ThrRS, respectively. Lane 4 (10-μl sample) shows the crude extract of the noninfected strain 159λind. Lanes 5 (5-μl sample) and 6 (10-μl sample) received crude extracts of strain 159λind infected with λp2. On lanes 7 and 8 samples (5 μl and 10, respectively) of an immunoprecipitate were applied, which was obtained by reacting crude extracts of λp2-infected cells (159λind) first with an anti-ThrRS serum and subsequently by precipitating the ThrRS-antibody complex with an anti-rabbit immunoglobulin serum from goats (Behringwerke, Marburg). On lanes 7 and 8 the two predominantly stained bands (from top) are the heavy and light chains of the immunoglobulins.*

with the biochemical and genetic information on the ThrRS described. The deoxyribonucleic acid of this phage carries a small wild-type *E. coli* chromosome segment coding for the α and β subunits of phenylalanyl-tRNA synthetase (8), for the ribosomal initiation factor IF3 (Springer et al., in press), and, as shown in this study, obviously also for the ThrRS. This indicates once again (i) the immediate neighborhood of the ThrRS and phenylalanyl-tRNA synthetase genes and (ii) the 76,000-dalton protein as being the sole constituent polypeptide of ThrRS, which appears to be responsible for the in vivo complementation of borrelidin-resistant enzymes in the *thrS8* strain lysogenic for λp2. Constituent polypeptides smaller than about 15,000 daltons, however, cannot be completely excluded, since the gel system used in the experiment of Fig. 5 does not reliably resolve proteins of that size.

It remains an interesting problem to determine how the transcription of the above-mentioned gene cluster coding for proteins with functions in the protein synthesis system is organized and regulated.

ACKNOWLEDGMENTS

We are very grateful to Heidi Franz, Margit Kaiser, Sigrid Nissen, and Gaby Tondl for their help and assistance. We thank Mathias Springer for many helpful suggestions, Ekkehard Holler for the gift of purified leucyl-tRNA synthetase and isoleucyl-tRNA synthetase, and Karl Poralla for the strain of *S. parvulus* and for the details concerning the isolation of borrelidin.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft to G.N.

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