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Biochemical and Immunological Characterization of Threonyl-tRNA Synthetase of Two Borrelidin-Resistant Mutants of *Escherichia coli* K12

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The threonyl-tRNA synthetase of two wild-type *Escherichia coli* K12 strains and of two borrelidin-resistant mutants thereof has been purified about 100-fold. A neutralizing antiserum has been prepared against one of the wild-type threonyl-tRNA synthetases. The comparative characterization of the threonyl-tRNA synthetases of the two wild strains and the two borrelidin-resistant mutants revealed that the threonyl-tRNA synthetase of one borrelidin-resistant mutant (K12B *bor*^{r} 2) exhibits a lowered K_m value for threonine and ATP and an increased K_i value for borrelidin with respect to threonine. Furthermore the activity of the threonyl-tRNA synthetase of this mutant is more sensitive to higher pH values, is more rapidly heat-inactivated and shows a different shape of its antiserum-neutralization curve than the threonyl-tRNA synthetase of the parental strain (K12B). The threonyl-tRNA synthetase of the other borrelidin-resistant mutant (K12B *bor*^{r} 3) was investigated in the same way and it did not show any difference to the threonyl-tRNA synthetase of the parental strain, except that the specific activity of the enzyme in crude extracts of this mutant is 5 times as high as the corresponding value in crude extracts of K12B.

From these data it is concluded that borrelidin resistance in K12B *bor*^{r}-2 is due to a structurally altered threonyl-tRNA synthetase (structural mutant) whereas in K12B *bor*^{r}-3 it is due to the constitutive increase of the threonyl-tRNA synthetase level by a factor of five (regulatory mutant).

The inhibition of the threonyl-tRNA synthetase by borrelidin with respect to threonine was noncompetitive in all four cases. In order to observe a considerable heat inactivation of the threonyl-tRNA synthetase within 10 min a temperature of 60 °C was necessary. The heat-inactivation process was not affected by the presence of threonine, but it was hastened by the presence of ATP or tRNA.

Borrelidin inhibits the growth of various organisms by inhibiting the enzymatic activity of their threonyl-tRNA synthetase [1]. Since no other biochemical reaction is known to be inhibited by borrelidin, there is a good chance that borrelidin-resistant mutants might exhibit an altered threonyl-tRNA synthetase activity. Therefore, borrelidin-resistant mutants of *Escherichia coli* K12 were isolated without using any mutagen; a preliminary characterization of the threonyl-tRNA synthetase of these mutants in crude extracts indicated the existence of some mutants with a structurally altered threonyl-tRNA synthetase and some mutants with an increased rate of formation of threonyl-tRNA synthetase (Nass, G. & Thomale, J., unpublished). In the present paper, we describe the verification of these observations by the partial purification and the characterization of the threonyl-tRNA synthetase of one $K_{m(app)}$ mutant, of one regulatory mutant and of the parental *E. coli* strain. Part of this work has been presented at the 7th FEBS meeting, Varna, 1971.

Enzymes. Threonyl-tRNA synthetase (EC 6.1.1.3); methionyl-tRNA synthetase (EC 6.1.1.10); glycyl-tRNA synthetase (EC 6.1.1.–); lysyl-tRNA synthetase (EC 6.1.1.6); arginyl-tRNA synthetase (EC 6.1.1.–); ribonuclease (EC 2.7.7.16); deoxyribonuclease (EC 3.1.4.5).

MATERIAL AND METHODS

Chemicals

All amino acids employed were the L-forms. Uniformly ^{14}C-labelled amino acids were obtained from New England Nuclear Corporation (Boston,

Mass.). *E. coli* B and *E. coli* K12 tRNA were purchased from Schwarz BioResearch (Orangeburg, N.Y.), ATP (dipotassium salt) was obtained from Mann Research (New York), DNAase I from Sigma Corp. (St. Louis, Mo.) and human albumin from Behringwerke (Marburg). The DEAE-cellulose was obtained from Whatman and the hydroxyapatite from Clarkson Chemical Co. Alcoa was a product of the Aluminium Co. (Arkansas).

Borrelidin was kindly provided by Dr K. Poralla and Dr H. Zähner.

Bacterial Strains and Growth Conditions

Wild-type strains with respect to threonyl-tRNA synthetase were: the RNAase-less *E. coli* K12 strain A19 [3] and the *E. coli* K12 strain K12B [4,5]. Two borrelidin-resistant mutants, K12B *bor*^{r}-2 and K12B *bor*^{r}-3, isolated without using any mutagen (Nass, G. & Thomale, J., unpublished), were used in this study. The threonyl-tRNA synthetase was purified from A19 cells, which were grown in rich medium by Fallek Products (New York) and from K12B, K12B *bor*^{r}-2 and K12B *bor*^{r}-3 cells grown by Merck AG (Darmstadt) in minimal medium enriched with 0.6% yeast extract. For small batches, growth was performed and followed in minimal medium as described earlier [6].

Purification of Threonyl-tRNA Synthetase

If not otherwise indicated, all purification steps were performed at 4 °C.

Preparation of Crude Extract. 100 g of frozen *E. coli* cells were thawed at room temperature and then homogenized at 4 °C with 200 g Alcoa in an automatic mill (RMO, Retsch, Haan, Germany) for 20 min. Then 200 ml buffer (0.01 M potasium phosphate pH 7.3 containing 10 mM magnesium acetate, 0.4 mM EDTA and 6 mM 2-mercaptoethanol) was added and the cells homogenized for another 20 min; then Alcoa and cell debris were removed by centrifugation (Sorvall, 30 min, 16000 rev./min, rotor SS 34) and 1000 units of DNAase were added to the cell-free extract. The ribosomes were then removed by centrifugation (Spinco L2 50B, Rotor 60 Ti, 3 h, 48000 rev./min) and glycerol was added to the supernatant to a final concentration of 10%.

Ammonium-Sulphate Precipitation. Saturated $(NH_4)_2SO_4$ solution in 0.01 M potassium phosphate buffer pH 7.3 containing 10% glycerol, 0.1 mM EDTA and 6 mM mercaptoethanol (buffer A), was added to the ribosome-free crude extract to reach a final saturation of 40%. Then a step-wise ammonium sulfate precipitation in 5% steps was performed up to 70% $(NH_4)_2SO_4$ saturation. Before assaying the threonyl-tRNA synthetase activity in the precipitated fractions they were dialysed for 24 h against buffer A.

The remaining steps in the purification of the threonyl-tRNA synthetase were performed essentially according to Hirsh [7].

DEAE-Cellulose Chromatography. The ammonium sulfate fraction with the highest specific activity of the threonyl-tRNA synthetase was applied to a DEAE-cellulose column (2×70 cm, equilibrated with buffer A). The column was eluted with a linear gradient of potassium phosphate (0.05 —0.4 M in buffer A; 1000 ml/1000 ml; 60 ml/h; 16-ml fraction size). The threonyl-tRNA synthetase activity of each fraction was determined and those with the highest activity were pooled and concentrated by dialysis against 3.6 M $(NH_4)_2SO_4$ in buffer A adjusted to pH 7.3 with ammonium hydroxide to a protein concentration of about 10 mg/ml. The resulting precipitate was dissolved in 0.01 M potassium phosphate buffer pH 6.8 containing 10% glycerol and 6 mM mercaptoethanol (buffer B) and dialysed against the same buffer.

Hydroxyapatite Chromatography. The concentrated, synthetase-containing fraction from the DEAE-cellulose chromatography was applied to a hydroxyapatite column (1×30 cm) equilibrated with buffer B. The column was eluted with a linear gradient of potassium phosphate (0.03—0.4 M in buffer B; 300 ml/300 ml; 20 ml/h; 9-ml fraction size). Fractions with the highest threonyl-tRNA synthetase activity were pooled and concentrated in an ultravacuum concentrator (Sartorius, Type SM 16305) until the protein concentration was greater than 1 mg/ml. The concentrated purified threonyl-tRNA synthetase was stored frozen at —80 °C in small portions until required. Under these conditions the threonyl-tRNA synthetase retained full activity for more than 18 months.

Assay of Threonyl-tRNA Synthetase

The activity of the threonyl-tRNA synthetase was measured by determining the rate of attachment of [^{14}C]threonine to tRNA [8,6]. The buffer for diluting the enzyme always contained 10% glycerol and, if not otherwise indicated, the pH of the Tris-HCl buffer was adjusted to pH 8.2 at 22 °C, leading to a pH during incubation at 37 °C of pH 7.85 (Sigma Chem. Co. Technical Bulletin No. 106 B). In some cases albumin was used in the incubation mixture and this is mentioned with the appropriate experiments. The substrate concentrations used during determination of enzyme constants are also listed with each experiment. One unit of enzyme activity is defined as the attachment of 1 μmol threonine to tRNA per hour. K_m values were determined according to the method of Lineweaver and Burk [9] and the K_i values according to Dixon [10].

Heat Inactivation

When investigating the heat stability of the threonyl-tRNA synthetase it was found that up to 60 °C there was no great loss of enzymatic activity after a 10-min incubation. Therefore, a temperature of 60 °C was used for the heat-inactivation experiments. Aliquots of the synthetase-containing fractions were diluted to a protein concentration appropriate for immediate assay of the threonyl-tRNA synthetase activity. However, before performing that enzyme assay, the aliquots were incubated for various times at 60 °C as described in each experiment and then chilled in ice before determining the threonyl-tRNA synthetase activity. This activity was compared with that of an untreated control sample which was kept in ice instead of being heated and the activity of which was taken as 100%. The percentage decline of the threonyl-tRNA synthetase activity during the heat treatment was calculated and then plotted against time in a semilogarithmic way.

Immunological Procedures

Preparation of antiserum to threonyl-tRNA synthetase was performed as described for the antiserum to phenylalanyl-tRNA synthetase [5]. As antigen, purified threonyl-tRNA synthetase of *E. coli* A19 cells was taken. A total of 2.2 mg protein of the purified enzyme was used. The antiserum did not show antibodies against any aminoacyl-tRNA synthetase other than threonyl-tRNA synthetase. The neutralization of the threonyl-tRNA synthetase activity by the antiserum was performed according to Cinader [11] and Pollock [12] by adding increasing amounts of antiserum to a constant quantity of enzyme as already described [5]. The threonyl-tRNA synthetase activity was measured in its aminoacylating reaction only.

Protein Concentration

This was determined by the standard colorimetric method [13], using human albumin as standard protein.

RESULTS

Table 1 shows the purification of threonyl-tRNA synthetase of *E. coli* K12B, the purification being about 90-fold. The threonyl-tRNA synthetases of *E. coli* A19, K12B *bor*^{r}-2 and K12B *bor*^{r}-3 were purified in a similar way. In the first series of experiments, the apparent K_m value for ATP and threonine of the purified threonyl-tRNA synthetases was determined; the results are sumarized in Table 2. The K_m values for ATP is identical for the threonyl-tRNA synthetase of *E. coli* A19, K12B and K12B *bor*^{r}-3, whereas the corresponding value for the threonyl-tRNA synthetase of K12B *bor*^{r}-2 is lower by a factor of two. The K_m value for threonine is about 84 μM for the threonyl-tRNA synthetase of *E. coli* A19, K12B and K12B *bor*^{r}-3 and 17 μM for the threonyl-tRNA synthetase of K12B *bor*^{r}-2. The K_m values for threonine of the threonyl-tRNA synthetase as determined in crude extracts of *E. coli* A19, K12B and K12B *bor*^{r}-3 were similar to the corresponding values found using purified enzyme preparations. However, the K_m value for threonine of the threonyl-tRNA synthetase in the crude extract of K12B *bor*^{r}-2 was 6 μM, thus being about three times lower than the value found using the purified enzyme from this mutant and 12 times lower than the corresponding value of the threonyl-tRNA synthetase from K12B, the parental strain of this mutant.

When investigating the inhibition of the purified threonyl-tRNA synthetase of K12B, K12B *bor*^{r}-2

Table 1. *Summary of the purification of the threonyl-tRNA synthetase of* E. coli *K12B*
Experimental details are listed under Material and Methods

Fraction	Volume	Protein	Total enzyme activity	Specific activity	Yield
	ml	mg/ml	U	U/mg protein	%
Ribosome-free crude extract	180.0	20.50	206.6	0.057	100
$(NH_4)_2SO_4$-precipitation (40—45%)	22.0	34.00	130.0	0.174	63
DEAE-cellulose	11.2	7.10	55.7	0.710	23
Hydroxyapatite	8.5	0.31	14.0	5.500	7

Table 2. *Michaelis-Menten constants of the purified threonyl-tRNA synthetase of various* E. coli *strains*
The threonyl-tRNA synthetase activity was determined as described under Material and Methods. However, when determining the K_m value for threonine, the threonine concentration was varied between 10 and 200 μM and when determining the K_m value for ATP the ATP concentration was varied between 25 and 200 μM final concentration. The protein concentration used per 0.5-ml incubation mixture was 0.26 μg for the threonyl-tRNA synthetase preparation of A19, 0.1 μg for threonyl-tRNA synthetase preparation of K12B, 0.1 μg for the threonyl-tRNA synthetase preparation of K12B *bor*^{r}-3 and 0.4 μg for the threonyl-tRNA synthetase preparation of K12B *bor*^{r}-2

E. coli strain	K_m value for	
	Threonine	ATP
	μM	μM
A19	83	120
K12B	85	120
K12B *bor*^{r}-3	82	110
K12B *bor*^{r}-2	17	50

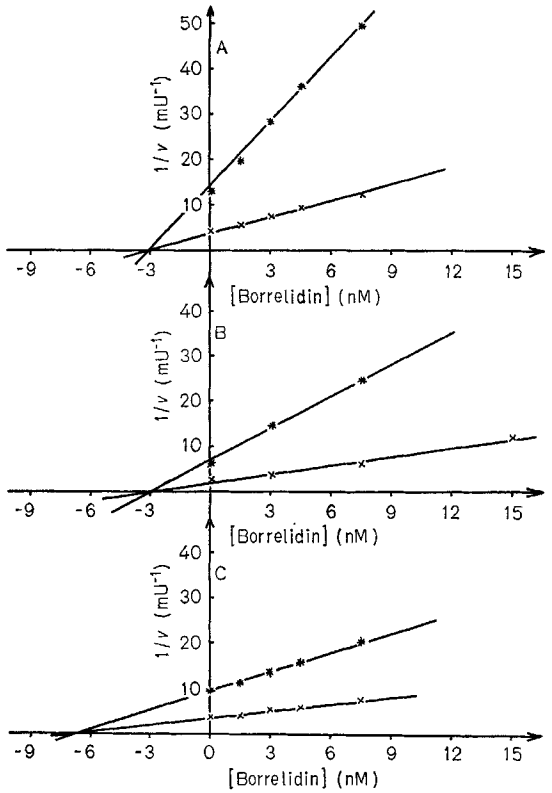

Fig. 1. *Determination of the inhibitor constant for borrelidin of the threonyl-tRNA synthetase in regard to threonine for various* E. coli *strains.* The constants were determined as described by Dixon [10]. The threonyl-tRNA synthetase activity was measured as described under Material and Methods. However, the incubation mixtures contained increasing amounts of borrelidin (0 to 15 nM final concentration). (*) Values when the final threonine concentration was 5 μM and (×) when using 20 μM threonine final concentration in the incubation mixture. The protein concentration in the incubation mixture of 0.5 ml was 0.1 μg of the purified threonyl-tRNA synthetase preparation of K12B (A) and K12B *bor*^{r}-3 (B) each and 0.5 μg of the purified threonyl-tRNA synthetase preparation of K12B *bor*^{r}-2 (C)

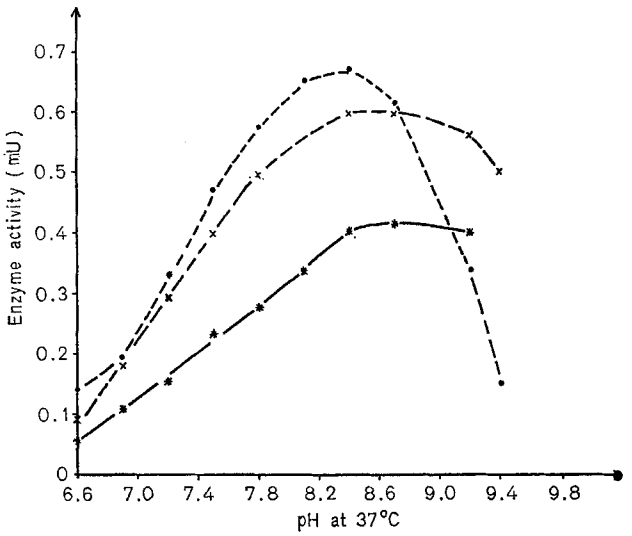

Fig. 2. *pH-dependency of the threonyl-tRNA synthetase activity.* The threonyl-tRNA synthetase activity was determined as described under Material and Methods. However, the pH of the Tris buffer in the incubation mixture was varied as indicated in the figure. The pH values of the Tris buffers were adjusted at 22 °C and the resulting pH values during incubation at 37 °C were calculated according to Sigma Chem. Comp. Technical Bulletin No. 106B. (×----×) pH dependency of the purified threonyl-tRNA synthetase of K12B, (*——*) of the purified threonyl-tRNA synthetase of K12B *bor*^{r}-3 and (●----●) of the purified threonyl-tRNA synthetase of K12B *bor*^{r}-2, the protein concentration of the purified enzyme preparations in the 0.5-ml incubation mixtures being 0.1 μg, 0.07 μg and 0.3 μg, respectively

and K12B *bor*^{r}-3 by borrelidin, in all three cases a non-competitive inhibition with respect to threonine was observed (Fig. 1), the resulting K_i values being 3.3 nM borrelidin for the threonyl-tRNA synthetase of K12B and K12B *bor*^{r}-3 and 7.5 nM borrelidin for the threonyl-tRNA synthetase of K12B *bor*^{r}-2. An identical pattern of inhibition of the threonyl-tRNA synthetase by borrelidin was observed in crude extracts of the two borrelidin-resistant mutants and the parental strain.

Fig. 2 shows that the pH optimum for the purified threonyl-tRNA synthetase of K12B and K12B *bor*^{r}-3 is rather broad, whereas the pH optimum for the purified threonyl-tRNA synthetase of K12B *bor*^{r}-2 is much sharper, although both center on pH 8.4. Also the activity of the threonyl-tRNA synthetase of the latter mutant declines much more rapidly when the pH of the incubation is increased above pH 8.7.

When investigating the heat stability of threonyl-tRNA synthetase at 60 °C, the time necessary for a 50% inactivation was shorter the purer the enzyme preparation became. Therefore, albumin was always added to the threonyl-tRNA synthetase samples before performing the heat inactivation to a final concentration of 2 mg/ml. This resulted in similar values for a 50% inactivation of threonyl-tRNA synthetase in crude extracts and in purified enzyme preparations from *E. coli* A19, K12B, and K12B *bor*^{r}-3, namely 12 min when incubating the enzyme preparations at 60 °C without substrates. The same time was necessary for the heat inactivation of the threonyl-tRNA synthetase in crude extract of K12B *bor*^{r}-2. However, in purified threonyl-tRNA synthetase preparations of this mutant, even in the presence of albumin, it took 8 min to inactivate 50% of the threonyl-tRNA synthetase activity (Fig. 3). The presence of either substrate during the heat treatment of the threonyl-tRNA synthetase resulted in a qualitatively similar picture for the

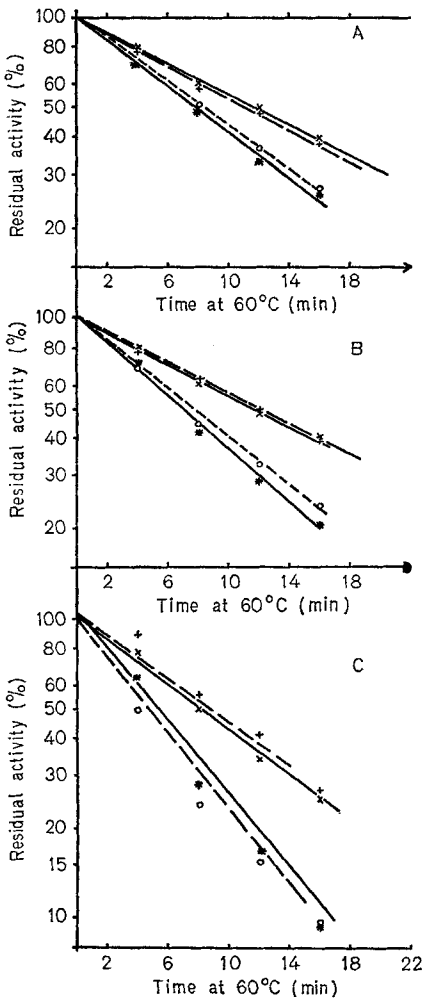

Fig. 3. *Heat inactivation of threonyl-tRNA synthetase of* E. coli *K12B, K12B* bor^r*-3 and K12B* bor^r*-2 in the absence and presence of substrates.* The heat treatment of the threonyl-tRNA synthetase in the purified enzyme preparations and the determination of threonyl-tRNA synthetase activity was performed as described under Material and Methods. When substrates were present during the heat treatment, the threonine concentration was 40 μM, the ATP concentration 4 mM and the tRNA concentration 5 mg *E. coli* B tRNA/ml which was determined to equal 2 μM $tRNA^{Thr}$. The protein concentration of the purified threonyl-tRNA synthetase preparation was 0.4 μg/ml during the heat treatment and 0.1 μg/0.5 ml in the enzyme assay for the threonyl-tRNA synthetase of K12B (A) and of K12B bor^r-3 (B). When assaying the heat stability of the purified threonyl-tRNA synthetase of K12B bor^r-2 (C), the protein concentration during the heat treatment was 1.2 μg/ml and 0.3 μg/0.5 ml in the enzyme assay, except during the heat inactivation of the threonyl-tRNA synthetase of this mutant in the presence of tRNA, where the protein concentrations were increased by a factor of two. (×——×) Heat inactivation of the threonyl-tRNA synthetase in the absence of substrates, (+----+) in the presence of threonine, (O----O) in the presence of ATP and (✱——✱) in the presence of tRNA

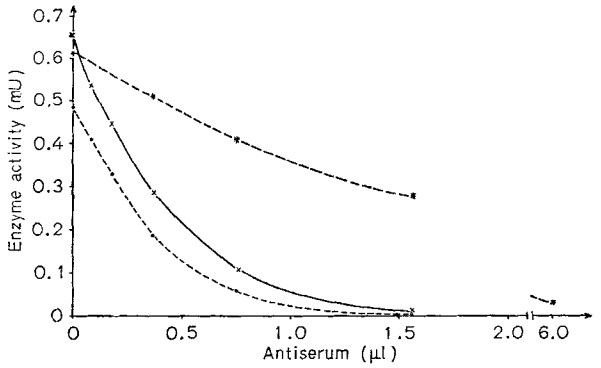

Fig. 4. *Antibody-neutralization curve for the threonyl-tRNA synthetase in crude extract of K12B, K12B* bor^r*-3 and K12B* bor^r*-2.* The preincubation of the crude extracts with the antiserum to threonyl-tRNA synthetase and the determination of the threonyl-tRNA synthetase activity were performed as described under Material and Methods. (×——×) Neutralization curve for the threonyl-tRNA synthetase of K12B (60 μg protein crude extract/0.6 ml preincubation mixture), (●----●) for the threonyl-tRNA synthetase of K12B bor^r-3 (15 μg protein crude extract/0.6 ml preincubation mixture) and (✱----✱) for the threonyl-tRNA synthetase of K12B bor^r-2 (35 μg protein crude extract/0.6 ml preincubation mixture)

threonyl-tRNA synthetase of K12B, K12B bor^r-3 and K12B bor^r-2 (Fig. 3): tRNA and ATP labilized the threonyl-tRNA synthetase in each case, whereas the presence of threonine did not influence the rate of heat inactivation.

The antibody-neutralization curve of the threonyl-tRNA synthetase in crude extracts of K12B bor^r-3 was similar to that obtained with K12B, whereas the curve obtained for the threonyl-tRNA synthetase of *E. coli* K12B bor^r-2 was shaped differently (Fig. 4): four times as much antiserum was necessary to neutralize the same amount of enzyme activity. The antibody-neutralization curves of the purified threonyl-tRNA synthetases of K12B and K12B bor^r-3 were identical to the ones obtained for the threonyl-tRNA synthetases in the corresponding crude extracts, indicating that no cross-reacting enzymatically inactive threonyl-tRNA synthetase was present in the purified enzyme preparations. However, in the case of K12B bor^r-2, the purified enzyme required five times as much antiserum as the crude extract to neutralize a given amount of threonyl-tRNA synthetase activity (Fig. 5), thus indicating the presence of enzymatically inactive or altered threonyl-tRNA synthetase in the purified threonyl-tRNA synthetase preparation of K12B bor^r-2.

DISCUSSION

Several times an altered structure of aminoacyl-tRNA synthetases has been demonstrated in partially purified enzyme preparations [14—18]. For the

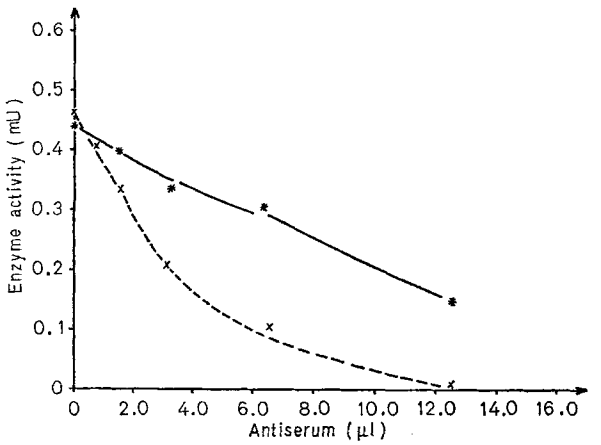

Fig. 5. *Antibody-neutralization curve for the purified threonyl-tRNA synthetase and for the threonyl-tRNA synthetase in crude extract of K12B* bor^r*-2.* The preincubation of the threonyl-tRNA synthetase in the purified enzyme preparation or in crude extract of K12B *bor*^{r}-2 and the determination of the threonyl-tRNA synthetase activity were performed as described under Material and Methods. (∗——∗) The neutralization curve for the threonyl-tRNA synthetase in the purified enzyme preparation (3.5 µg protein of the purified enzyme preparation/0.6 ml preincubation mixture) and (×----×) for the threonyl-tRNA synthetase in the crude extract (50 µg protein crude extract/0.6 ml preincubation mixture)

characterization of the threonyl-tRNA synthetases of two *E. coli* K12 wild strains and two borrelidin-resistant mutants thereof, we used about 100-fold purified threonyl-tRNA synthetase preparations. During all purification steps of the threonyl-tRNA synthetase from the four different *E. coli* K12 cells, always only one peak of threonyl-tRNA synthetase activity was observed, indicating the presence of only one kind of threonyl-tRNA synthetase in the cells.

The threonyl-tRNA synthetase of *E. coli* B had earlier been partially characterized, the K_m value for ATP and threonine being 100 µM and 12 µM, respectively [7]. The K_m value for ATP of the threonyl-tRNA synthetase from *E. coli* K12B and A19 were similar to the one reported for *E. coli* B, but the K_m value for threonine was found to be 85 µM. The difference in the latter enzyme constant could be due to the different *E. coli* strains, like the differences known for some animoacyl-tRNA synthetases of *E. coli* B and K12 [19], or to the different buffer systems used during the determination of the threonyl-tRNA synthetase activity, as it is known that enzyme constants of aminoacyl-tRNA synthetases can vary according to assay conditions [20, 21].

The relatively high temperature of 60 °C was necessary for inactivating the threonyl-tRNA synthetase to a considerable extent within 10 min at pH 7.5, the temperature for the heat inactivations of other aminoacyl-tRNA synthetases being around 45 °C [22, 15, 23, 24]. The presence of tRNA or ATP during heat treatment resulted in a more rapid loss of threonyl-tRNA synthetase activity of both the wild types and the borrelidin-resistant mutants, but the substrate threonine was without any influence upon the heat inactivation process. So far a heat labilization of aminoacyl-tRNA synthetases by homologous tRNA has only been reported for lysyl-tRNA synthetase [25] and arginyl-tRNA synthetase [26] of rat liver. In all other cases investigated the tRNA has been found to be protective [15, 21, 25, 26] or without any effect [23, 25, 27] upon the heat inactivation of aminoacyl-tRNA synthetases. A labilization of the complex between ATP and threonyl-tRNA synthetase of rat liver by homologous tRNA had been shown by gel-filtration studies [28]. The heat labilization of an aminoacyl-tRNA synthetase by the presence of the substrate ATP has now been demonstrated for the threonyl-tRNA synthetase but has not been observed so far for other aminoacyl-tRNA synthetases [15, 21, 27]. The reported labilization of the threonyl-tRNA synthetase by ATP and tRNA indicates the induction of a conformational change of threonyl-tRNA synthetase by these substrates.

The characterization of the threonyl-tRNA synthetase of *E. coli* K12B *bor*^{r}-2 revealed differences to the wild-type threonyl-tRNA synthetase in all parameters investigated: the K_m value for threonine is lowered by a factor of about 12 in the crude extract of this mutant and by a factor of 4 in the purified enzyme preparation, the K_m value for ATP is lowered by factor of 2 and the K_i value for borrelidin in regard to threonine is increased by a factor of about 3. Furthermore, the threonyl-tRNA synthetase of this mutant loses its enzymatic activity more rapidly at pH values above 8.4 than the parental threonyl-tRNA synthetase does, less time is necessary to inactivate the threonyl-tRNA synthetase at 60 °C in the absence or presence of substrates and about 30-fold the amount of antiserum is necessary to neutralize a given amount of threonyl-tRNA synthetase activity from this mutant when compared with the parental threonyl-tRNA synthetase. All these parameters were also determined for the purified threonyl-tRNA synthetase of *E. coli* K12B *bor*^{r}-3 and all the values were identical to the ones obtained for the wild-type threonyl-tRNA synthetase.

From these data we conclude that borrelidin resistance in *E. coli* K12B *bor*^{r}-2 is due to a structurally altered threonyl-tRNA synthetase. Since the threonyl-tRNA synthetase of *E. coli* K12B *bor*^{r}-3 behaves during all investigations like the threonyl-tRNA synthetase of the wild type, and since the specific activity of the threonyl-tRNA

synthetase of this mutant in crude extracts is five times the one in crude extract of the parental strain, the borrelidin resistance in *E. coli* K12B *bor*^{r}-3 is achieved by the presence of five times the amount of structurally unchanged threonyl-tRNA synthetase.

A constitutive three-fold increased level of wild-type methionyl-tRNA synthetase has been observed in a certain *E. coli* K12 strain, though the molecular event or selecting agent responsible for this increase is not known [29]. Folk and Berg [30] found a 2 to 4-fold increase of the specific activity of structurally altered glycyl-tRNA synthetase in revertants of their glycyl-tRNA synthetase mutants; the increase of the specific activity is supposed to be due to gene duplication [30]. In our *E. coli* K12B *bor*^{r}-3 mutant the level of wild-type threonyl-tRNA synthetase is increased by a factor of five. The selecting agent for this constitutive increase of threonyl-tRNA synthetase activity was borrelidin. Evidence will be presented that about 30% of our borrelidin-resistant mutants of *E. coli* K12 show a 4—6-fold constitutively increased level of structurally unaltered threonyl-tRNA synthetase (Nass, G. and Thomale, J., unpublished). These mutants are now being further investigated in order to find the molecular devices regulating the formation or destruction rate of threonyl-tRNA synthetase in *E. coli* K12 cells.

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