ALTERATION OF RIBOSOMAL PROTEIN L5 IN A THIOPEPTIN-RESISTANT MUTANT OF ESCHERICHIA COLI

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Summary: Chromatographic analysis of ribosomal protein components of a thiopeptin-resistant mutant of E. coli on a carboxymethyl-cellulose column showed that one of 50S ribosomal proteins was altered. In two-dimensional electrophoresis, protein L5 was deficient in the 50S ribosomal subunit of the thiopeptin-resistant mutant. In reconstitution experiments, the resistance was localized in the core of ethanol-NH_4Cl-treated 50S subunits or in the split protein of 4.8 M CsCl-treated 50S subunits. The results indicate that the thiopeptin resistance may be attributed to alteration of ribosomal protein L5.

Thiopeptin, a sulfur-containing peptide antibiotic, inhibits both elongation factors (EF)-G- and Tu-associated functions by acting on the 50S ribosomal subunit (1,2). Siomycin and thiostrepton have been reported to exhibit a similar effect (3-9). A thiopeptin-resistant mutant has been isolated, and the resistance has been shown to be attributed to alteration of the 50S ribosomal subunit (2). It has been also demonstrated that both EF-T-dependent binding of aminoacyl-tRNA to the mutant ribosomes and complex formation of the mutant ribosomes with EF-G and GDP are resistant to the antibiotic (2). In the further experiments, it has been observed that the resistance is associated with alteration of ribosomal protein L5 of the mutant 50S subunit, and the results are presented in this communication.

Materials and Methods

Thiopeptin-resistant mutants were obtained from E. coli Q13 by treatment with N-methyl-N'-nitro-N-nitrosoguanidine (2). [^{3}H]GDP (620 mCi/mmole) was a product of the Radiochemical Centre, Amersham, England. Elongation factor (EF)-G was prepared by the method of Nishizuka et al. (10). EF-G-

dependent binding of [^{3}H]GDP to the ribosomes in the presence of fusidic acid was examined by a similar procedure as described previously (11,12). Carboxymethyl-cellulose chromatography was performed by the method of Tanaka et al. (13). The particles of 50S ribosomal subunits were reconstituted with core and split protein fractions, which were produced by treatment of the 50S subunits with 1 M NH_4Cl and 50% ethanol by the technique of Sander et al. (14). The extraction with 4.8 M CsCl and the reconstitution followed the procedure of Meselson et al. (15). The method of Kaltschmidt and Wittmann (16) was applied to the two-dimensional polyacrylamide gel electrophoresis, using the apparatus of YEDA Research and Development Co., Rehovot, Israel.

Results

The mechanism of thiopeptin resistance was studied with a resistant mutant of E. coli Q13, in which the resistance was localized in the 50S ribosomal subunits (2). The 30S and 50S ribosomal proteins of the parent strain were labelled with [^{3}H]lysine, and those of the thiopeptin-resistant mutant with [^{14}C]lysine, following the method described previously (17). The dissociated 30S or 50S ribosomal proteins from the two organisms were mixed and chromatographed on a carboxymethyl-cellulose column. No difference was detected in the patterns of 30S ribosomal proteins of these two strains. However, in the 50S ribosomal proteins, a peak, as indicated with an arrow in Fig. 1, was deficient in the thiopeptin-resistant mutant. The other peaks of [^{14}C] and [^{3}H]lysine labels were found at the identical positions (Fig. 1).

The patterns of 50S ribosomal proteins of the sensitive and resistant cells were compared by the two-dimensional polyacrylamide gel electrophoresis. The resistant mutant was lacking in L5 spot, while all the other spots showed good correspondence in both strains (Fig. 2).

The 50S ribosomal subunits from the thiopeptin-sensitive and -resistant cells were treated with 1 M NH_4Cl and 50% ethanol. The extract and core from both origins were mixed and the 50S particles were reconstituted. The complex formation of the reconstituted 50S subunits with EF-G and GDP was investigated in the presence of fusidic acid. From the results presented in Table 1, the thiopeptin resistance was found to be localized in the

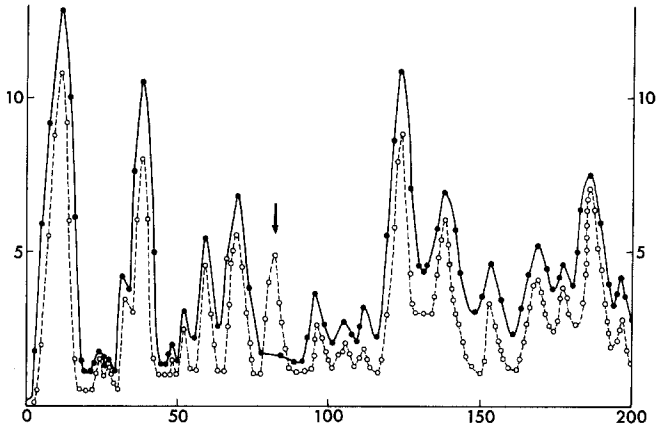

Fig. 1. Co-chromatography on a carboxymethyl-cellulose column of labelled 50S ribosomal proteins from thiopeptin-sensitive and -resistant cells of E. coli.

---o---o---o--- x 10^3cpm [^{3}H]lysine-incorporated 50S proteins of the parent strain

—●—●—●— x 10^2cpm [^{14}C]lysine-incorporated 50S proteins of the thiopeptin-resistant mutant

core, which contained L5; but not in the extract (L7 and L12).

The 50S subunits were treated with 4.8 M CsCl, and reconstituted; and the activity of complex formation with EF-G and GDP was examined in a similar way. The thiopeptin resistance was detected in the split protein fraction, which included protein L5 (Table 2).

The results suggested that the thiopeptin resistance may be attributed to alteration of ribosomal protein L5.

Discussion

From the effect of thiopeptin on the ribosomal functions, we have indicated a close relationship of the 50S ribosomal sites which interact with EF G and EF-Tu (1,2). It has been shown that the 50S ribosomal proteins L7 and L12 are required for EF G-dependent GTPase reaction (18), and that the extraction of ribosomal proteins L7 and L12 by ethanol-NH_4Cl treatment affects various functions associated with EF-G and EF-Tu (19,20). However,

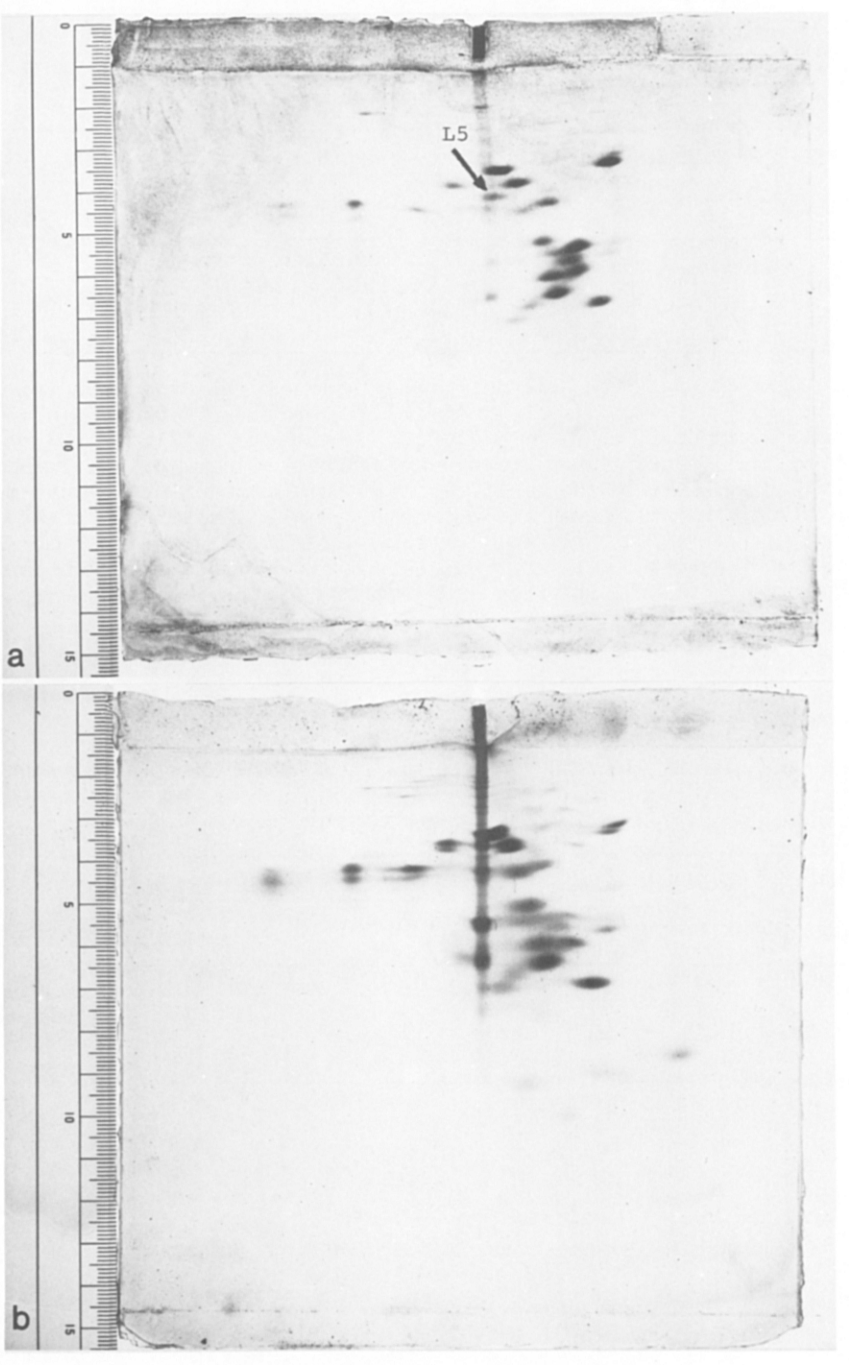

Fig. 2. Two-dimensional polyacrylamide gel electrophoresis of 50S ribosomal proteins.
(a) the parent strain (b) the thiopeptin-resistant mutant

Table 1. Localization of the thiopeptin resistance in the core of NH_4Cl-ethanol-treated 50S ribosomal subunits: Reconstitution of the 50S ribosomal subunits obtained from thiopeptin-sensitive and -resistant cells.

30S subunits	Reconstituted 50S subunits		pmoles [^{3}H]GDP bound to ribosomes		
			concentration of thiopeptin 10^{-7} M		
	SP	core	0	10	20
S	S	S	24.2(100)	12.4(51.2)	9.4(38.3)
S	S	R	19.3(100)	15.3(79.2)	12.7(70.1)
S	R	S	17.9(100)	10.4(57.8)	8.5(47.2)
S	R	R	20.1(100)	17.5(87.0)	15.4(76.5)

The reaction mixture in 0.1 ml contained: 50 mM Tris-HCl, pH 7.4, 10 mM $Mg(AcO)_2$, 60 mM NH_4Cl, 2 mM dithiothreitol, 200 μg core, 40 μg split protein (SP), 6 μg EF G, 18 nmoles [^{3}H]GDP and 0.25 mM fusidic acid. After incubation at 18°C for 10 minutes, 2 ml of ice-cold buffer (10 mM Tris-HCl, pH 7.4, 10 mM $Mg(AcO)_2$, 60 mM NH_4Cl, and 2 mM dithiothreitol) containing 0.2 mM fusidic acid was added. [^{3}H]GDP bound to the ribosomes was collected on Millipore filters, and washed with fusidic acid-containing buffer; and the radioactivity was counted. The number in the bracket represents per cent of control.

Table 2. Localization of the thiopeptin resistance in the split proteins of 4.8 M CsCl-treated 50S ribosomal subunits: Reconstitution of the 50S subunits obtained from thiopeptin-sensitive and -resistant cells.

30S subunits	Reconstituted 50S subunits		pmoles [^{3}H]GDP bound to ribosomes		
			concentration of thiopeptin 10^{-7} M		
	SP	core	0	10	20
S	S	S	23.4(100)	14.0(59.8)	9.3(39.7)
S	S	R	22.1(100)	15.1(68.3)	10.2(46.2)
S	R	S	20.2(100)	16.1(80.1)	13.8(68.3)
S	R	R	19.6(100)	16.3(83.2)	14.1(71.9)

The reactions were performed as described in Table 1, except that 200 μg core and 80 μg split protein (SP) of 4.8 M CsCl-treated 50S ribosomal subunits were employed.

ribosomes lacking in L7 and L12 have been observed to bind thiostrepton(2

Maassen and Möller (22) have observed by photo-affinity labeling that ribosomal proteins L5, L11, L18, and L30 are involved in EF-G-dependent GDP binding. Highland et al. (23) have reported that ribosomal protein L11 is required for the binding with thiostrepton. We have indicated in

this communication that the thiopeptin resistance may be attributed to alteration of ribosomal protein L5. It remains to be determined whether L5 directly participates in the binding with thiopeptin or the change of L5 influences the binding of thiopeptin to other ribosomal protein(s). From the studies on the mode of action of thiopeptin and the mechanism of resistance, we suggest that ribosomal protein L5 may be one of specific proteins required for the functions associated with EF-G and EF-Tu.

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