Molec. gen. Genet. 169, 345–347 (1979)

MGG

Short Communication

Analysis of Lincomycin Resistance Mutations in *Escherichia coli*

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Summary. High level lincomycin resistant strains of *Escherichia coli* were isolated and screened for altered ribosomal proteins and functions. Amongst 58 strains investigated by electrophoresis one had an altered ribosomal protein S7, another one a mutated L14 and two showed altered L15 proteins.

A correlation between these alterations and lincomycin resistant growth could not be demonstrated by genetic analysis for any of the mutants. In vitro, however, extracts from the two L15 mutants were less sensitive to inhibition by the drug. A gene locus (*lin*^{R}) responsible for the lincomycin resistance phenotype was mapped at min 30 of the *Escherichia coli* chromosome near *tyrR*; it seems to be identical to the previously described *linB* locus (Apirion, 1967); however, in contrast to these reports it does not seem to alter any ribosomal function.

Introduction

Lincomycin inhibits protein synthesis in gram-positive and – to a lower degree – also in gram-negative bacteria (for review see Vazquez, 1974; Pestka, 1977). In *Escherichia coli* the antibiotic is reported to block translation of polyA [1] and polyC (Vazquez, 1966; Teraoka et al., 1969) whereas somewhat contradictory results exist whether or not it inhibits polyU-directed polyphenylalanine synthesis (Vazquez, 1966; Chang et al., 1966; Apirion 1967). Lincomycin was shown to bind to the 50S subunits of bacterial ribosomes (Chang et al., 1966) and to act on aminoacyl-tRNA binding and the transpeptidation reaction (Monro and Vazquez, 1967; Igarashi et al., 1969; Celma et al., 1970). Its action is antagonistic to that of the antibiotic erythromycin (Vazquez, 1974; Pestka, 1977).

A gene locus (*linB*) for high level lincomycin resistance was roughly mapped between *proB* and *his* on the *E. coli* chromosome (Bachmann et al., 1976) and evidence was presented that it affects a ribosomal function (Apirion, 1967). Until now, no detailed information concerning the identity of any altered ribosomal component in lincomycin-resistant strains was reported.

Results and Discussion

Spontaneous and EMS-induced mutants resistant to 1 mg lincomycin per ml were isolated from the parental strains HfrH (Hfr, *thi-1, rel-1, λ*$^-$) CSH57 (F$^-$, *ara, leu, lacY, purE, gal, trp, his, argG, malA, strA, xyl, mtl, ilv, metA(B), thi*) and PR11 (genotype as CSH57, in addition, *aroE353, spc-13*). Whereas the minimal inhibitory plate concentration of these strains is 200 μg/ml for lincomycin, those of the mutants obtained are in the range between 1 and 5 mg per ml. 58 independent isolates were tested for altered ribosomal proteins by two-dimensional (2-D) polyacrylamide gel electrophoresis (Kaltschmidt and Wittmann, 1970). Three derivatives of HfrH showed alterations: mutant LE2-10 had a changed S7 protein (not shown), strain LE2-12 a more acidic protein L14 (Fig. 1B) and LE2-7 a protein L15 which apparently comigrates with protein L14. The same migration pattern of L15 was also exhibited by a derivative of PR11, namely mutant LE5-1 (Fig. 1C). All four mutants were isolated after mutagenesis with EMS.

Parallel to the electrophoretic analysis of ribosomal proteins, S30 extracts from 67 lincomycin resis-

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[1] Abbreviations: polyC = polycytidylic acid; polyA = polyadenylic acid; polyU = polyuridylic acid; tRNA = transfer ribonucleic acid; EMS = ethylmethane-sulfonate; lin^R, str^R, spc^R = phenotypically resistant to lincomycin, streptomycin or spectinomycin, respectively

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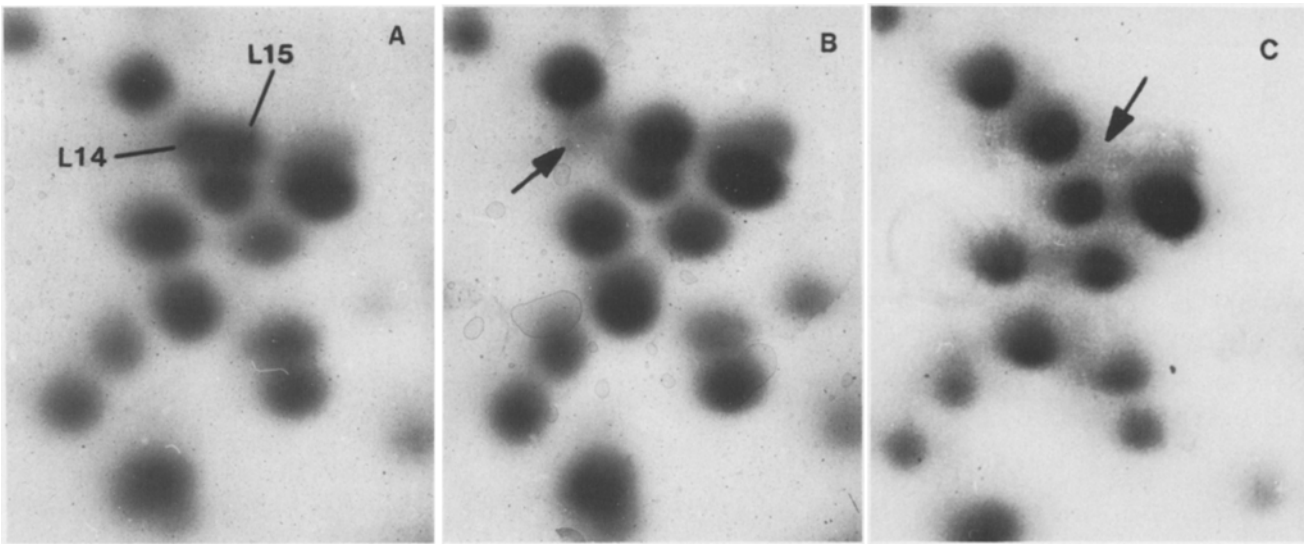

Fig. 1 A–C. Sections of 2-D electropherograms of total ribosomal proteins. **A** HfrH; **B** LE2-12, the mutant L14 protein is indicated by arrow; **C** LE5-1, the normal position of protein L15 is given by arrow. The intensity of the L14 spot indicates that the mutant L15 migrates into the L14 position

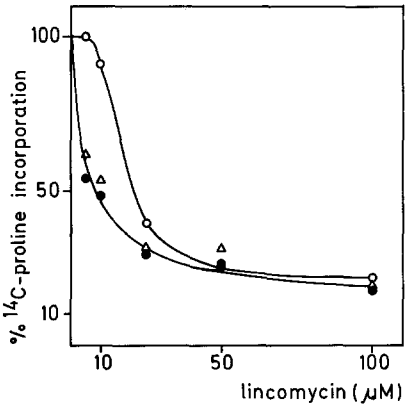

Fig. 2. Effect of different lincomycin concentrations on polyC-directed incorporation of [^{14}C]proline. The test system of Nierhaus et al. (1973) was used with some modifications. A total volume of 0.15 ml contained 12 mM Tris/HCl ph 7.8, 36 mM/NH_4Cl, 30 mM KOH, 12 mM magnesium acetate, 1.3 mM ATP, 0.04 mM GTP, 6.45 mM phosphoenolpyruvate, 3 mM mercaptoethanol, 0.05 mM [^{14}C]proline (10 μCi/μMole), 3 μg pyruvate kinase, 40 μg polyC, 40 μg *Escherichia coli* tRNA, 2 to 4 A_{260} units of an extract centrifuged at 30,000 g for 30 min (=S30). (●)HfrH; (△)LE2-12 (L14-mutant); (○)LE2-7 (L15-mutant)

tant strains were assayed for polyC-directed polyproline synthesis in the presence of the antibiotic. [The polyU system was not suitable for this determination due to its insensitivity to lincomycin, a finding which is in accordance with results reported by Vazquez (1966)]. Extracts from all mutants tested were still sensitive to lincomycin, the majority being inhibited as severely as that from the sensitive parental strain [Fig. 2, strain LE2-12 (L14 mutant which also is *linB*, see below)]. On the other hand, there is a distinct and reproducible effect of the L15 mutations in strains LE5-1 and LE2-7 on lincomycin inhibition at concentrations of the antibiotic lower than 20 μM (Fig. 2). Another lincomycin-resistant mutant strain L2/8-2, could not be inhibited to less than 40% residual activity even at high antibiotic concentrations; however, this strain did not show any visible alteration of a ribosomal protein. All three strains with altered in vitro sensitivity of polyproline synthesis to lincomycin also exhibit a reduced basic translational activity in the absence of the antibiotic in comparison to wild-type extracts.

The results obtained in the polyC system could be supported by the determination of the effect of lincomycin on the R17 RNA dependent incorporation of [^{14}C]valine. This test system, too, is sensitive to lincomycin and the results obtained were essentially the same as with polyC as messenger. For instance, 25 μM lincomycin reproducibly inhibited incorporation of [^{14}C]valine by wildtype ribosomes to 32%, that by the two L15 mutants to 52 and 54% residual activity, respectively.

The correlation between the alteration of ribosomal proteins and in vivo resistance was analysed by transductional analysis. Since the structural genes for proteins S7, L14 and L15 are situated near *aroE*, *aroE*$^+$ from a spontaneous *aroE*$^+$ derivative of strain LE5-1 was transduced (Miller, 1972) into strain AB2834 (F$^+$, *thi-21, mal-352, aroE353*) and from strains LE2-7, LE2-8 and LE2-12 into strain AB2834 *str*^{R} *spc*^{R}. By gel electrophoretic analysis of several recombinants from each cross it was verified that,

the mutations are cotransducible with *aroE* and, therefore, seem to be structural gene mutations. Out of 100 transductants tested in each experiment none was able to grow on plates containing 500 μg or 1 mg of lincomycin per ml. One has to conclude therefore that none of the ribosomal mutations observed is connected with the increased in vivo resistance.

To genetically localize the gene conferring lincomycin resistance *lin*^{R} we have chosen the L14 mutant the ribosomes of which are fully sensitive. Analysis of recombinants from a mating between LE2-12 and CSH57 roughly indicated a location of *lin*^{R} between *trp* and *his*, more close to the *trp* site. Attempts to cotransduce *lin*^{R} with *trpA* and *aroD* failed, but two out of 88 transductants became *lin*^{R} in addition to *tyrR*$^-$ (p-aminophenylalanine resistance) when this latter phenotype was transduced from a spontaneous lincomycin-resistant mutant of strain AB3271 (Wallace and Pittard, 1969) into a *tyrR*$^+$ (p-aminophenylalanine sensitive) derivative of the same strain. This suggests that the mutation conferring high level lincomycin resistance in strains LE2-12 and in strain AB3271 *lin*^{R} may be identical to the *linB* mutation described by Apirion (1967) and suggests a position for it close and clock-wise to *tyrR* (Bachmann et al., 1976).

In summary, one can state that it was not possible to isolate one-step mutants in which the target site of lincomycin has become high-level resistant; this indicates that more than one protein contributes to lincomycin binding to the ribosome. It is interesting that ribosomes from the two L15 mutants are more resistant to lincomycin in vitro though this increase of resistance is not reflected in the growth response to the drug. Protein L15 has been implicated recently in the formation of the A-site and the peptidyltransferase center (Czernilofsky et al., 1974; San José et al., 1976) which are functions of the ribosome reported to be affected by lincomycin in vitro (Monro and Vazquez, 1967; Igarashi et al., 1969; Celma et al., 1970; Vazquez, 1974; Pestka, 1977). Furthermore, L15 is reported to contribute to erythromycin binding (Teraoka and Nierhaus, 1977) and there is evidence that erythromycin and lincomycin posses overlapping binding sites at the ribosome (Vazquez, 1974; Pestka, 1977).

High level resistance to lincomycin, on the other hand, is conferred by mutations mapping close to *tyrR*, which (in the L15 mutants) may act cooperatively with the ribosomal mutations. They are possibly identical with the *linB* mutations described by Apirion (1967). The biochemical basis of their contribution to resistance is not yet known; in contrast to previous reports (Apirion, 1967) we could not detect any alterations in the ribosomal structure or function which are directly accounted for by the lincomycin-resistance. The results therefore stress the importance of genetic analysis for the demonstration of a correlation between a certain phenotype and the alteration of a cellular component.

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Communicated by H.G. Wittmann

Received October 3 / November 13, 1978