CHARACTERIZATION OF AN AMINO-ACID SUBSTITUTION LEADING TO SPECTINOMYCIN RESISTANCE IN *ESCHERICHIA COLI*

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We have reported [1] the identification of the 30 S ribosomal protein (P4) conferring sensitivity to the amino-glycoside antibiotic spectinomycin (Spc) by reconstitution techniques and have since shown that the P4 protein of Spc^R mutants may be distinguished from the P4 protein of a wild type strain by ion-exchange chromatography of the labelled proteins [2, 3]. The P4 protein from a Spc^R strain differs significantly from normal P4 in net electric charge since it elutes on carboxy-methyl cellulose columns at a lower ionic strength than normal P4.

We now show that amino-acid composition and finger print analysis can characterize the amino-acid substitution caused by the mutation to spectinomycin resistance. 30 S ribosomes from strains MRE600 and MRE600 Spe^R_{803} were isolated by zonal centrifugation and their proteins extracted following RNase digestion [4]. The P4 proteins, isolated on carboxy-methyl cellulose columns [5], were more than 95% pure, as estimated on acryl-amide gels. Each sample was then hydrolyzed with HCl and the amino-acid composition determined; this is shown in table 1. Each value is the average of three separate analyses. It is apparent that the amounts of lysine and glutamic acid residues are significantly different in the sensitive and resistant cases. However, since glutamic acid and glutamine are indistinguishable in our procedure, we cannot tell which one of the two amino-acids replaces lysine in the P4 (Spc^R_{803}) protein. According to the genetic code, either of these amino-acid substitutions satisfies a single base change. It is known however [6] that the mutator gene, discovered by Treffers [7], which produces specific adenine-thymine → cytosine-guanine transversions, cannot induce mutations to spectinomycin resistance. It is thus very likely that the transversion A→ C leading from lysine to glutamine is impossible and that the inserted amino-acid in the P4 protein of the Spc^R_{803} mutant is a glutamic acid.

Finger-prints analyses were performed for both P4 (Spc^S) and P4 (Spc^R_{803}) proteins. After tryptic digestion, the samples were chromatographed on thinlayer plates of cellulose powder in a two dimensional system using elution mixtures of butanol, acetic acid, water 3:1:1 and pyridine, isobutyric acid, isopropanol, water 1:1:2:1. Fig. 1. shows a

Table 1
Amino acid composition of the P4 proteins from parent strain MRE 600 Spc^S and mutant strain MRE 600 Spc^R_{803}.

Amino acid	P4 Spc^S (%)	P4 Spc^R_{803} (%)	Δ
Asp	9.7	10.1	+ 0.4
Thr	3.8	3.9	+ 0.1
Ser	12.4	12.0	− 0.4
Glu	*15.0*	*16.0*	*+ 1*
Gly	14.2	14.3	+ 0.1
Ala	8.3	8.0	− 0.3
Val	8.6	8.7	+ 0.1
Met	1.3	1.4	+ 0.1
Ile	3.9	3.9	0
Leu	6.7	7.1	+ 0.4
Tyr	1.7	1.7	0
Phe	3.0	3.1	+ 0.1
His	1.7	1.7	0
Lys	*8.5*	*7.3*	*− 1.2*

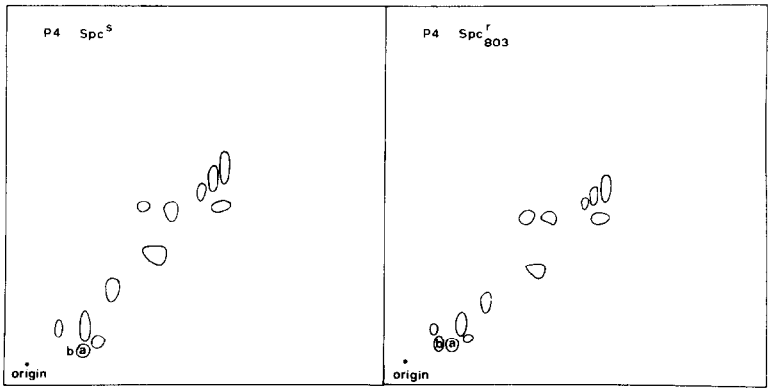

Fig. 1. Finger printing analysis of P4 proteins from MRE 600 Spc^S and MRE 600 Spc$^R_{803}$. Spot (a) decreases sharply in intensity in the mutant P4 finger print. Spot (b) appears in the mutant P4 finger print.

schematic representation of the finger prints. The major differences between wild type P4 and mutant P4 consist of a new spot (b) in P4 (Spc$^R_{803}$) finger print with a corresponding decrease in the intensity of another one (a).

We conclude from these results that the mutation Spc^S to Spc$^R_{803}$ results probably in the change of a lysine into a glutamic acid in the 30 S ribosomal protein P4. This modification is presumably responsible for the change in the properties of the 30 S ribosomal subunit. Further studies are in progress to determine the role of the P4 protein in the 30 S ribosome.

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