THE RIBOSOMAL PROTEIN ALTERED IN SPECTINOMYCIN RESISTANT *ESCHERICHIA COLI*

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In a previous paper [1] using reconstitution experiments [2] we reported the identification of the 30 S ribosomal protein conferring sensitivity to the antibiotic spectinomycin in *Escherichia coli.* The present work shows that the mutation to spectinomycin resistance (spc^r) significantly alters one particular protein of the 30 S moeity. This modification is revealed by chromatographic analysis of several independent spc^r mutants.

We studied the 30 S ribosomal proteins isolated from the following bacterial strains:

(1) *Escherichia coli* strain B and the B spc^r_9 and B spc^r_{10} spontaneous mutants isolated by Anderson [3].

(2) *Escherichia coli* strain MRE 600 (RNAase) and the spontaneous mutant spc^r_{803} isolated by us.

The cells were labelled with either ^{14}C or ^{3}H amino acids, harvested, then mixed to form the

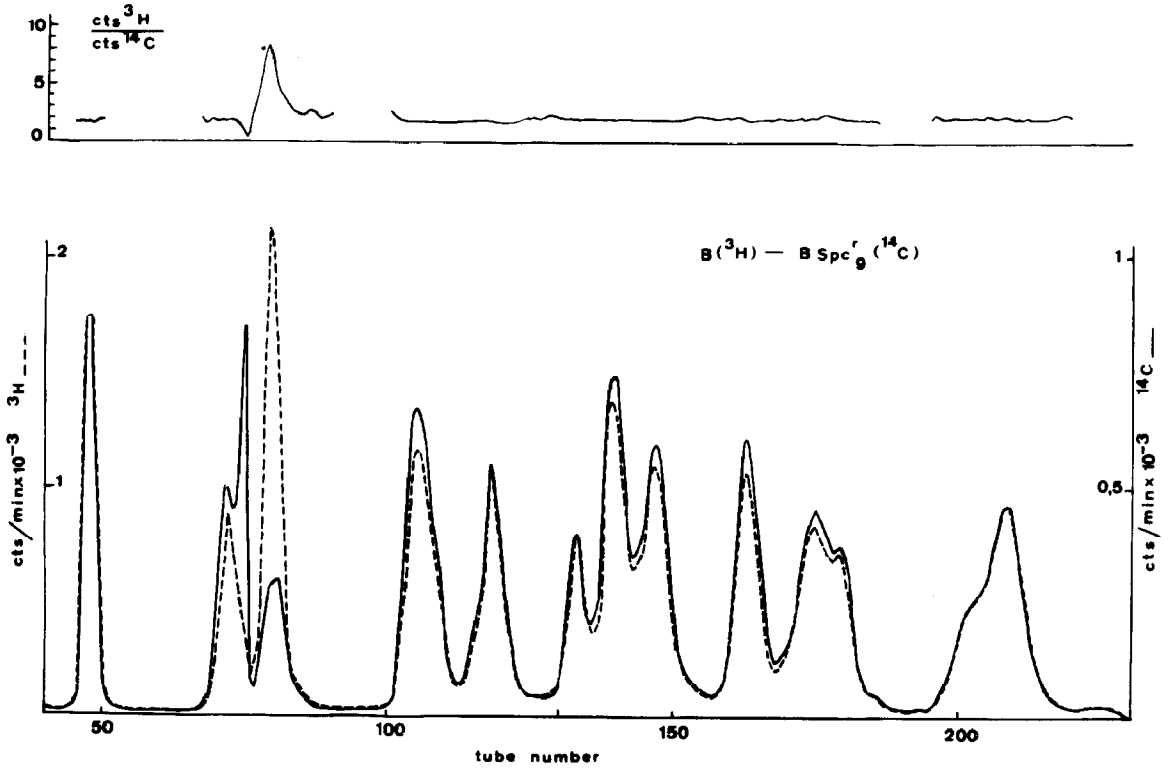

Fig. 1.

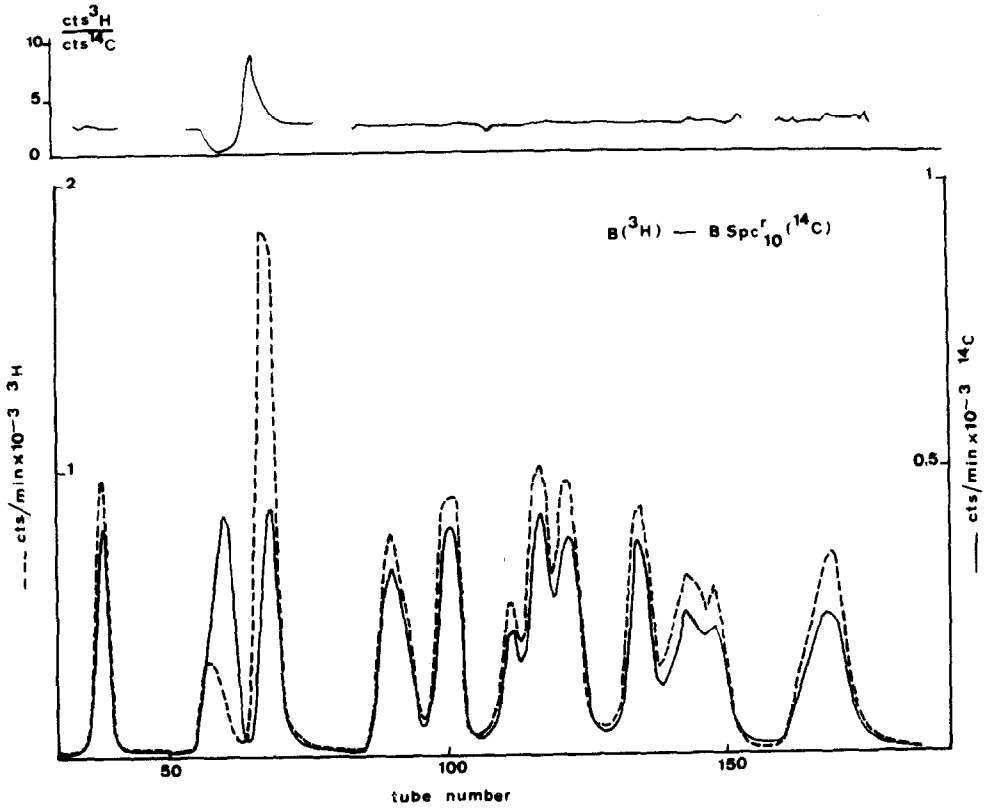
cts ^{3}H / cts ^{14}C
10
5
0
2
1
B(^{3}H) — B Spc$^{r}_{10}$(^{14}C)
--- cts/min x10^{-3} ^{3}H
— cts/min x10^{-3} ^{14}C
1
0.5
50
100
150
tube number

Fig. 2.

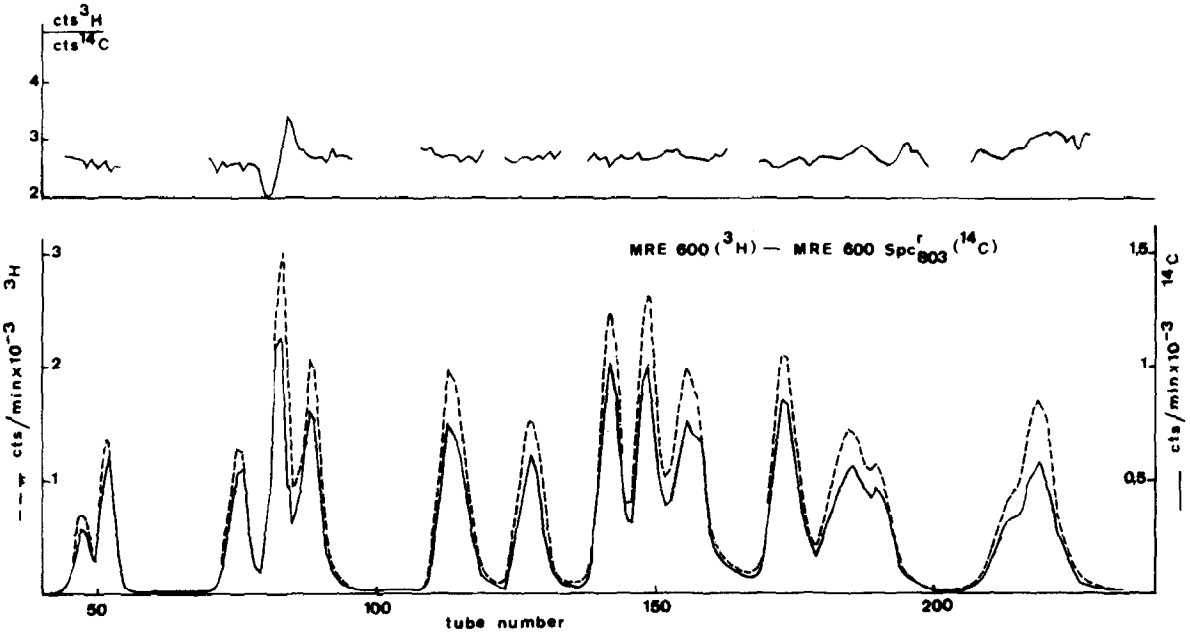
cts ^{3}H / cts ^{14}C
4
3
2
MRE 600 (^{3}H) — MRE 600 Spc$^{r}_{603}$ (^{14}C)
--- cts/min x10^{-3} ^{3}H
— cts/min x10^{-3} ^{14}C
3
2
1
1.5
1
0.5
50
100
150
200
tube number

Fig. 3.

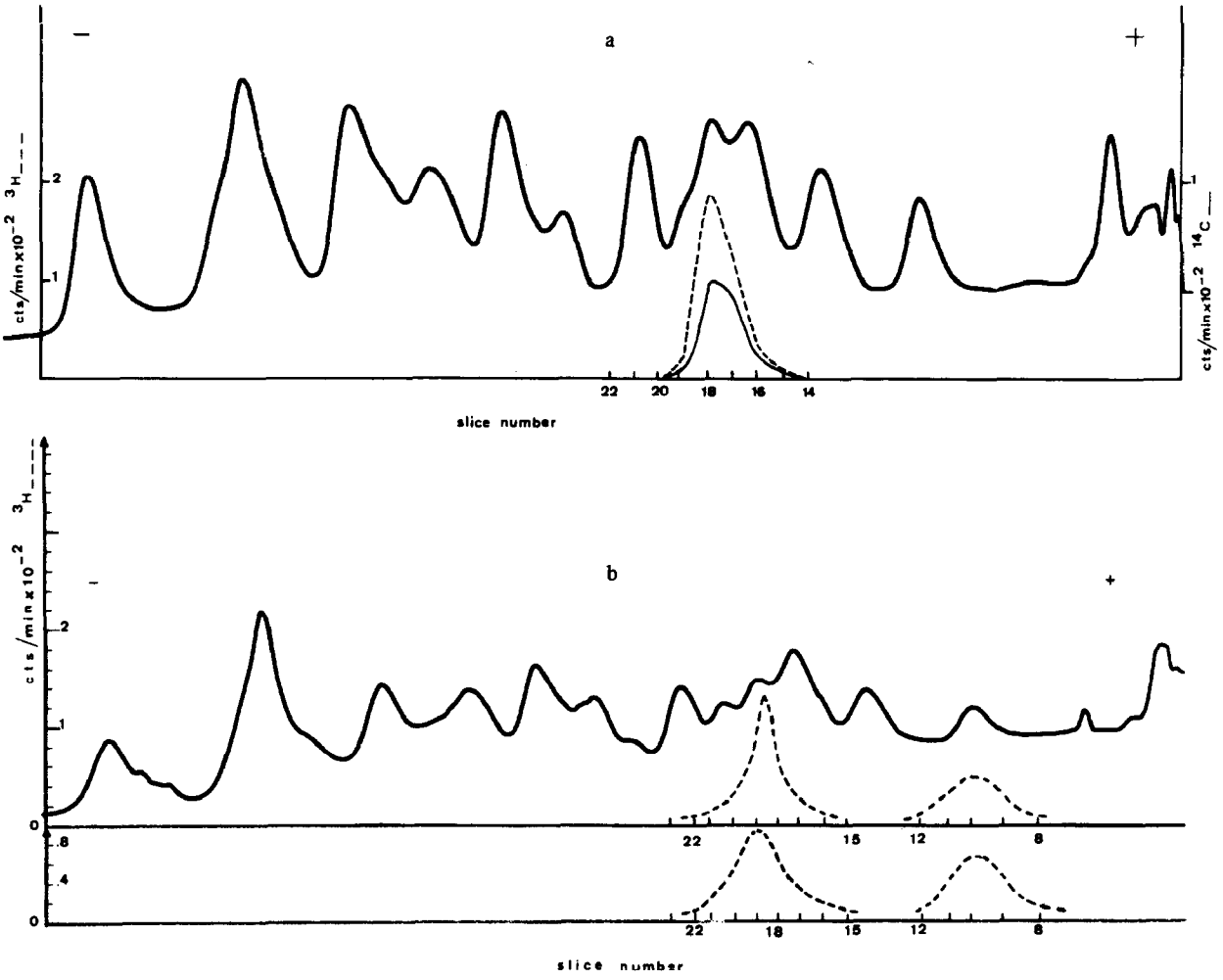

Fig. 4.

required pairs. The bacteria were ground with a French pressure cell and the 70 S ribosomes isolated as described by Capecchi [4]. The 30 S subunits were obtained by density gradient centrifugation and digested with pancreatic T_1 RNAases in a methylamine acetate buffer 0.03 M pH 5.6 containing 6 M urea, 3 mM β-mercaptoethanol and 0.25 mM EDTA.

Samples were applied on a 0.6 X 17 cm CMC column equilibrated in the digestion buffer without EDTA. The proteins were eluted with a linear gradeint from 0 to 0.4 M Na acetate in the same buffer. The ^3H and ^{14}C activities were measured by liquid scintillation in a toluene-triton X 100 mixture [5].

Figs. 1 and 2 show the chromatographic patterns for the mixtures of 30 S ribosomal proteins obtained from B and B spc^r_9, and B and B spc^r_{10} respectively. The two diagrams reveal the same type of difference in one of the early eluting proteins, this difference being still more evident looking at the ratio of the ^3H to the ^{14}C counts.

Fig. 3 represents the pattern obtained for the mixture MRE 600/MRE 600 spc^r_{803}; although the difference is not directly apparent, it is clearly revealed by the sharp variation in the ratio of the ^3H to the ^{14}C counts.

In order to further identify the spectinomycin protein, we performed an electrophoretic analysis involving a mixture of cold total proteins from the spc^s and spc^r 30 S and material from the altered region of the chromatograms. The gels were scanned

for the stain, then sliced and counted for the activity.

In fig. 4a, the activity is clearly completely located under one single band of the gel pattern displayed by fraction 83 from the MRE 600/MRE 600 spc^r_{803} mixture. This band corresponds to the protein labelled P_4 by Ozaki et al. [6] ; 8_a by Moore et al. [7] and 3 by Hardy et al. [8].

In fig. 4b, the upper part of the activity diagram corresponds to the fraction 79 from the B/B spc^r_9 mixture and the lower part to the fraction 68 from the B/B spc^r_{10} mixture. A major peak of activity can be seen under the P_4 band and a minor component corresponding to Ozaki's P_2, Hardy's 4a and Moore's 11 species. This protein is clearly a contaminant of P_4 in the chromatography of B/B spc^r_9 and B/B spc^r_{10} whereas it was separated in the MRE 600/MRE 600 spc^r_{803} chromatography. Recently, in a footnote, Takata et al. [9] reported observations on spc^r mutants altered in the protein labelled 30-4 which, as judged by its position in the chromatogram, corresponds to the protein we identified.

Acknowledgements

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