STREPTOMYCIN- AND VIOMYCIN-INDUCED CONFORMATIONAL CHANGES OF RIBOSOMES DETECTED BY IODINATION

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1. Introduction

The aminoglycoside antibiotic streptomycin binds to the 30 S ribosomal subunit and affects several ribosomal functions (reviewed [1,2]). It causes phenotypic suppression in vivo, misreading in cell-free systems programmed with synthetic polynucleotides, inhibition of polypeptide chain elongation directed by natural mRNA, destabilization of initiation and elongation complexes and inhibition of ribosomal dissociation. Streptomycin induces a conformational modification of the ribosomal structure [3–6], but the ribosomal proteins affected by this change have not been identified. Much less is known about the basic peptide antibiotic viomycin. It inhibits both the binding of fMet-tRNA$_F$ to the 30 S subunit and the translocation step of polypeptide chain elongation [7]. Thus, there is a binding site for viomycin on the 30 S subunit. Viomycin does not induce misreading [8].

In the present communication we report the use of protein iodination catalyzed by the enzyme lactoperoxidase to identify ribosomal proteins whose conformation and/or chemical environment is modified by the binding to the ribosome of these antibiotics. The validity of this method for the study of structural modifications of the ribosome has already been established [9,10]. We have found that the binding of either streptomycin or viomycin to 70 S ribosomes from *Escherichia coli* modifies the iodination of at least 5 or 9 ribosomal proteins, respectively.

2. Materials and methods

The preparation of 1 M NH_4Cl-washed *E. coli* MRE 600 ribosomes has been described [11]. Bovine lactoperoxidase (EC No. 1.11.1.7.) and glucose oxidase (EC No. 1.1.3.4.) were purchased from Sigma Chemical Co. The lactoperoxidase was further purified by filtration through Sephadex G-100 [12]. $Na^{125}I$ (11–17 mCi/μg I) was from the Radiochemical Centre, Amersham. Viomycin sulphate was a gift from Parke-Davis Co.

Prior to use, ribosomes (145 A_{260} units/ml) were activated by incubating them at 30°C for 30 min in 70 mM NH_4Cl, 20 mM $Mg(acetate)_2$, 10 mM Tris–HCl, pH 7.8. Binding of antibiotics to ribosomes was carried out in mixtures (100 μl) containing: 70 mM NH_4Cl, 10 mM $Mg(acetate)_2$, 10 mM Tris–HCl, pH 7.8, 72 A_{260} unit/ml activated ribosomes and 0.1 mM of either streptomycin or viomycin. Incubation was at 30°C for 10 min. ^{125}I incorporation was performed by supplementing the mixtures containing antibiotic-treated ribosomes, or parallel mixtures with untreated ribosomes (final vol. 120 μl), with 10 μg/ml lactoperoxidase, 0.2 mM $Na^{125}I$ (150–300 cpm/pmol), 0.4 mM D-glucose and 7 μg/ml glucose oxidase. The ionic conditions were identical to those for the binding of antibiotics. After incubation at 30°C for 1 h, the incorporation was stopped by addition of 50 mM 2-mercaptoethanol and incubation at 30°C for 10 min. Samples (5 μl) were precipitated with cold 5% trichloroacetic acid to determine the amount of ^{125}I incorporated. To the remainder of the incorporation mixtures, 50–58 A_{260} units of carrier ribosomes were added and the ribosomal proteins were extracted with 69% acetic acid and precipitated with acetone [13]. Two dimensional gel electrophoresis was performed as described [14]. Gel slabs were stained with Coomassie brilliant blue in 3.5% perchloric acid [15],

the protein spots were cut out and their radioactivity was determined by gamma scintillation counting.

3. Results

Under the iodination conditions used in our experiments each 70 S ribosome incorporated on average between 39 and 67 atoms ^{125}I. In spite of this difference, the distribution of ^{125}I among the ribosomal proteins (table 1) was reproducible in 7 different experiments performed with 2 independent preparations of ribosomes. However, it differs from iodination patterns reported [9,10,16], probably due to our different experimental conditions.

Streptomycin and viomycin did not appreciably affect the total incorporation of ^{125}I by 70 S ribosomes (not shown). However, table 2 shows that 0.1 mM streptomycin changed the extent of iodination of several ribosomal proteins. Incorporation into proteins S10, S12 + L20, and L10 was diminished whereas that into L17 and L28 was increased. Incorporation into proteins S13 + L24 was significantly changed, but the variation was very small. Iodination

Table 1
Incorporation of ^{125}I into ribosomal proteins in intact 70 S ribosomes

Protein	Incorporated radioact. (%)	Protein	Incorporated radioact. (%)
S1	2.40 ± 0.35	L1	1.18 ± 0.15
S2	1.57 ± 0.20	L2	7.58 ± 0.67
S3	8.97 ± 1.38	L3	1.19 ± 0.17
S4	3.36 ± 0.28	L5	3.49 ± 0.19
S5 + L6	2.70 ± 0.27	L9	1.62 ± 0.40
S7	3.83 ± 0.38	L10	4.99 ± 0.60
S8	0.92 ± 0.18	L11	7.08 ± 0.50
S9 + S11	13.4 ± 0.7	L13	3.88 ± 0.26
S10	0.33 ± 0.09	L14	1.96 ± 0.15
S12 + L20	1.81 ± 0.23	L15	3.34 ± 0.24
S13 + L24	1.59 ± 0.07	L16	0.60 ± 0.09
S14	0.74 ± 0.06	L17	2.43 ± 0.27
S15 + S16 + S17	1.75 ± 0.34	L18	1.32 ± 0.32
S18	1.85 ± 0.37	L19	0.50 ± 0.05
S19	1.53 ± 0.13	L21	0.38 ± 0.16
S20	1.10 ± 0.13	L22	1.27 ± 0.12
S21	0.51 ± 0.14	L23	0.47 ± 0.06
		L25	0.96 ± 0.15
		L27	2.36 ± 0.50
		L27′	0.61 ± 0.08
		L28	0.32 ± 0.08
		L29	0.44 ± 0.20
		L30	0.48 ± 0.15
		L32	0.80 ± 0.26

The results, expressed as mean values ± SEM, are averages of 7 experiments performed as described in section 2. Total radioactivity (100%) recovered from each two-dimensional gel varied from 123 000–357 000 cpm. Most of this variation is due to the use of $Na^{125}I$ of different specific activities and to differences in the incorporation of ^{125}I by ribosomes (see text), rather than to changes in the recovery of radioactivity during extraction and analysis of ribosomal proteins, which was always very close to 25%. L27′ refers to a protein that moves in the electrophoretic field as L27 in the second dimension, and trails behind it in the first. Data for proteins S6, L4, L7, L8, L12, L31, L33 and L34 are not shown since the recovery of these proteins varied in the different experiments and incorporation into them was always very low

Table 2
Effect of 0.1 mM streptomycin on the incorporation of ^{125}I by ribosomal proteins

Protein	Incorporation (% recovered radioact.)	$\bar{r}$	p
S10	0.19 ± 0.06	0.63 ± 0.26	0.975–0.99
S12 + L20	1.37 ± 0.11	0.77 ± 0.08	>0.995
S13 + L24	1.45 ± 0.10	0.91 ± 0.05	0.99–0.995
L10	3.95 ± 1.11	0.78 ± 0.15	0.975–0.99
L17	2.95 ± 0.41	1.23 ± 0.19	0.95–0.975
L28	0.47 ± 0.15	1.51 ± 0.37	0.975–0.99

Incorporations, expressed as mean values ± SEM, are averages of 7 experiments performed as described in section 2. $\bar{r}$ is the mean value of the ratios of incorporation in the presence of antibiotic (streptomycin) to incorporation in its absence obtained for each protein in each of the 7 experiments. The probability of $\bar{r}$ being different from 1 is indicated by the value of p in the student's t significance test. Proteins with $p < 0.95$ were considered to have $\bar{r}$ values that did not significantly differ from 1 and have been omitted from the table

of most of the remaining proteins (not shown) was found not to be significantly modified.

Table 3 shows the results of similar experiments carried out in the presence of 0.1 mM viomycin. Incorporation into proteins S4, S8 and L1 was diminished; incorporation into S7, S18, S21, L5, L27′ and L28 was increased. The variations in the degree of iodination of proteins S9 + S11, L3, L13 and L22 were small and, consequently, difficult to interpret.

4. Discussion

The binding of either streptomycin or viomycin to the ribosome modifies the iodination of at least 5 or 9 ribosomal proteins, respectively. These considerable effects do not seem to be caused by non-specific binding of the drugs promoted by their polycationic character, since each antibiotic modifies the labelling of a different set of proteins. Moreover, the changes of label-

Table 3
Effect of 0.1 mM viomycin on the incorporation of ^{125}I by ribosomal proteins

Protein	Incorporation (% recovered radioact.)	$\bar{r}$	p
S4	2.85 ± 0.46	0.85 ± 0.11	0.95–0.975
S7	4.47 ± 0.76	1.17 ± 0.09	0.975–0.99
S8	0.65 ± 0.13	0.72 ± 0.21	0.95–0.975
S9 + S11	14.8 ± 0.9	1.09 ± 0.06	0.95–0.975
S18	3.11 ± 0.76	1.88 ± 0.57	0.95–0.975
S21	0.83 ± 0.29	1.62 ± 0.23	>0.995
L1	0.94 ± 0.16	0.81 ± 0.12	0.975–0.99
L3	1.01 ± 0.10	0.87 ± 0.09	0.95–0.975
L5	4.40 ± 0.32	1.25 ± 0.11	0.99–0.995
L13	3.37 ± 0.21	0.89 ± 0.08	0.95–0.975
L22	1.13 ± 0.18	0.88 ± 0.06	0.975–0.99
L27′	0.82 ± 0.26	1.96 ± 0.70	0.95–0.975
L28	0.53 ± 0.12	1.39 ± 0.25	0.95–0.975

Incorporations are averages of 5 experiments performed as described in section 2. Meanings of $\bar{r}$ and p are explained in the legend to table 2. Proteins with $p < 0.95$ have been omitted from the table

ling in so many proteins may well be correlated with the multiple effects of these antibiotics on ribosomal function (see section 1).

Streptomycin clearly modifies the iodination of proteins S10, S12 + L20, L10, L17 and L28. The 30 S proteins in this group have been implicated in the action of streptomycin. Protein S12 has been shown to control the characteristics of the sensivity, resistance or dependence of the ribosome with respect to the antibiotic (reviewed [17]). Specific antibodies against proteins S10 and S12 inhibit the binding of streptomycin to the 30 S subunit [18]. The change in the iodination of 50 S proteins may result from an influence on the 50 S subunit of the conformational alteration of the 30 S subunit. In this regard, it is of interest that protein S12 is thought to be located in the interface between the ribosomal subunits [19]. An alternative explanation, however, is that streptomycin may bind to the 50 S subunit [20], specially at the relatively high concentration used in this study, and directly affect its conformation.

While it is clear that viomycin binds to the 30 S subunit [7], it is not ruled out that it may also interact with the 50 S subunit, for multiple-step selection procedures carried out with *Mycobacterium smegmatis* have made possible the isolation of viomycin-resistant mutants having either modified 30 S or modified 50 S subunits [21]. Our results show that viomycin clearly alters the iodination of S4, S7, S8, S18, S21, L1, L5, L27′ and L28. With regard to its action on the 30 S subunit, it is of interest that at least part of proteins S7 and S21 appear to be located near the 3′-end of 16 S RNA [22,23]. Moreover, proteins S4 and S7 can be crosslinked to S8, and S18 to S21 [24], and antibodies against proteins S18 and S21 inhibit fMet-tRNA binding to 30 S subunits [18]. Thus, viomycin evidently modifies the conformation of a region of the 30 S subunit around the 3′-end of 16 S RNA, an effect that probably explains its ability to inhibit fMet-tRNA binding. This same distortion may also be responsible for the inhibition of translocation by the antibiotic. However, it is suggestive that viomycin modifies the labelling of proteins L1 and L5. Protein L5 is close to the interaction site of elongation factor (EF) G [25] and is modified in mutants resistant to thiopeptin [26] (an antibiotic that prevents interaction of EF-G and aminoacyl-tRNA with the ribosome [1,2] and there are indications (A. Zamir, personal communication) that protein L1 reacts with a derivative of erythromycin (an antibiotic that under some conditions inhibits translocation [27]). Thus, it is also possible that the alteration of these proteins, or of nearby ribosomal components, may account for the inhibition of translocation by viomycin.

There are conflicting reports on the modification of gross ribosomal structure by streptomycin. Studies on the reactivity of the ribosomal RNA to kethoxal and spin-labelling experiments suggest a 'loosening' of the ribosomal structure [5,6], while studies using hydrogen–tritium exchange indicate the opposite [3,4]. Our results show that streptomycin, and also viomycin, increase the susceptibility to iodination of some proteins and decrease that of others. This suggests that the drugs do not cause a general 'loosening' or 'tightening' of ribosomal structure. Instead, they probably induce conformational rearrangements of several proteins and possibly rRNA that result in the masking of some regions of these ribosomal components and in the exposure of others. Thus, depending upon the specificity of the technique employed a 'loosening' or a 'tightening' of the ribosomal structure may be apparent. The conformational changes induced by these drugs may be quite subtle such that techniques capable of discrimination at the level of individual atoms or residues may prove the most appropriate for their study.

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