BINDING OF AUREOVOCIN TO RIBOSOMES OF *STREPTOMYCES AUREOFACIENS* B-96

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1. Introduction

Evidence has been reported for the interaction of several antibiotic compounds with ribosomes. *Streptomyces aureofaciens* produces tetracycline antibiotics which inhibit protein synthesis in mammalian cell-free systems [1, 2] and bacterial cells [3, 4]. It has been suggested [5, 6] that the mechanism of the action of tetracyclines is related to their binding to ribosomes which causes the inhibition of transfer of aminoacyl t-RNA to ribosome-m-RNA complexes.

Aureovocin, a new glucosidic compound with a chinoic nucleus, has been isolated from *S. aureofaciens* [7]. The present paper reports the *in vivo* binding of Aureovocin to ribosomes of the cells which produce this substance.

2. Materials and methods

Streptomyces aureofaciens non-tetracycline-producing strain (B-96) and tetracycline-producing strain (84/25) were obtained from the Research Institute of Antibiotics and Biotransformations (Prague). The cultures were grown at 28° C in 500 ml flasks containing 60 ml media with 3% sucrose, 1% $(NH_4)_2SO_4$, 0.01% mixture of aminoacids, 0.05% NaCl, 0.05% K_2HPO_4, 0.05% $MgSO_4$, 0.001% $ZnSO_4$, 0.008% $MnCl_2$ and 0.1% yeast extract, pH = 7.0.

The cells were harvested after 10 hr and washed by suspension in a cold buffer containing 10 mM Tris-HCl, pH 7.5, 10 mM magnesium acetate, 60 mM KCl and 6 mM β-mercaptoethanol (buffer I). The washed cells were ground with alumina (Reanal) and extracted with buffer I (1 ml/mg cells). Cell fragments and alumina were removed by centrifugation at 20.000 × g for 20 min. The supernatant was treated with 2 μg/ml of DNase (Worhington, electrophoretically purified) for 20 min at 5° C. Ribosomes were prepared from the extracts by differential centrifugation [8].

The ribosomes were further washed twice with

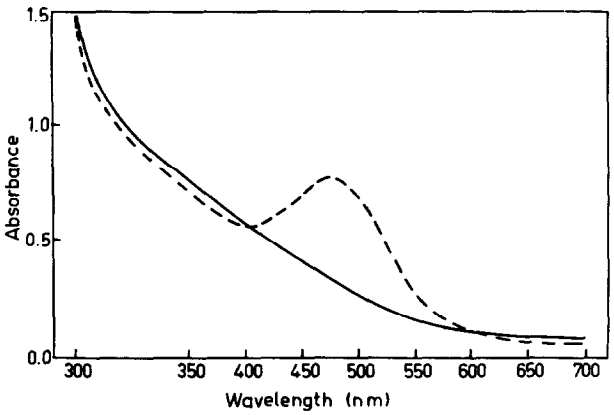

Fig. 1. The visible spectra of ribosomes of *S. aureofaciens* isolated from 10 hr cultures of tetracycline producing (——) and non-tetracycline-producing (- - -) strains. Ribosomes of both strains (1 mg/ml) were resuspended in buffer I.

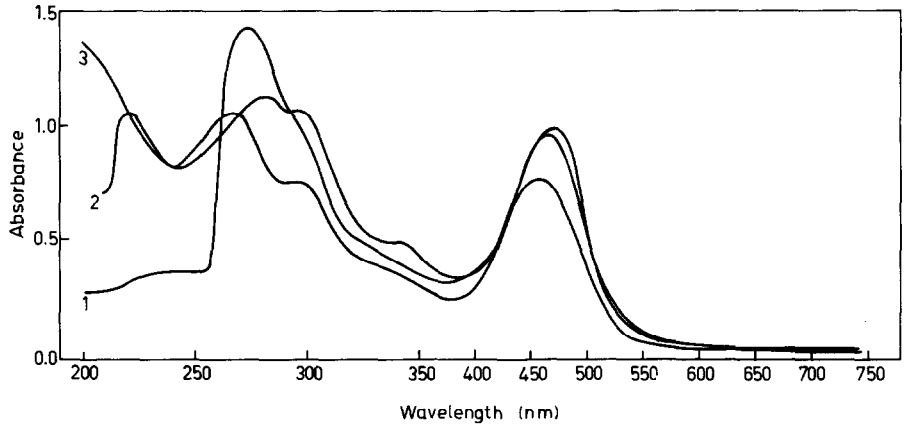

Fig. 2. The spectra of the Aureovocin extracted from ribosomal pellet of non-tetracycline-producing strain. 1 – in dimethylsulphoxide, 2 – in buffer I, 3 – in formamide.

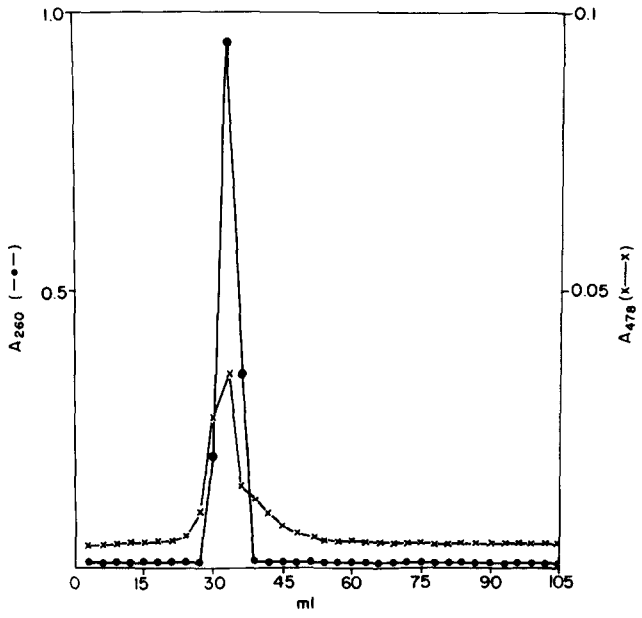

Fig. 3. Elution profile of the ribosome-Aureovocin complex. Two mg of ribosomes were applied to a Sephadex G-50 column (2 X 50 cm) and eluted with buffer I at 2° C. Individual fractions (3 ml) were collected. A_{260} (– . –) of r-RNA and A_{478} (– x –) of the Aureovocin ribosome complex.

buffer and three times with 0.5M $(NH_4)_2SO_4$, then dialyzed against buffer I for 16 hr and stored at -20° C as a pellet.

All spectra were recorded with a Perkin-Elmer 402 spectrophotometer using a 1 cm silica cuvette.

Gel filtration was performed in the cold through Sephadex G-50 (Pharmacia, Uppsala, Sweden) in columns (2 X 50 cm).

3. Results and discussion

Fig. 1 shows the visible spectra of ribosomes isolated from 10 hr cultures of tetracycline (TC) producing strain (84/25) and non-TC-producing mutant (B-96) of *S. aureofaciens*. The ribosomes of non-TC-producing strain bind the orange pigment, which absorbs at 478 nm. It was impossible to remove this compound from the ribosomes by washing with 0.5M $(NH_4)_2SO_4$ or 0.5M KCl. To identify the properties of the pigment, the ribosomal pellet was extracted with dimethylsulphoxide. The extract was chromatographed in a solvent system containing chloroform, butanol, McIlvaine buffer pH 4.5 (4:1:5) [9]. The red spot of R_F = 0.37 was eluted and its spectra recorded in different solvents. Fig. 2 shows the spectral properties of the pigment in dimethylsulphoxide (λ_{max} = 276 and 468), formamide (λ_{max} = 282, 299, 345 and 476) and buffer I (λ_{max} = 276, 298 and 458). All spectra and R_F

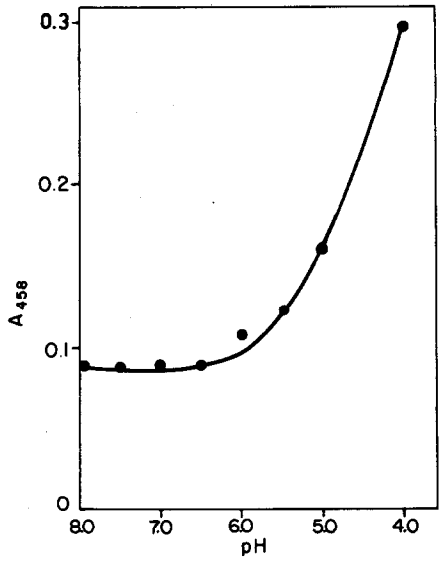

Fig. 4. Stability of the Aureovocin-ribosome complex at different pH's.

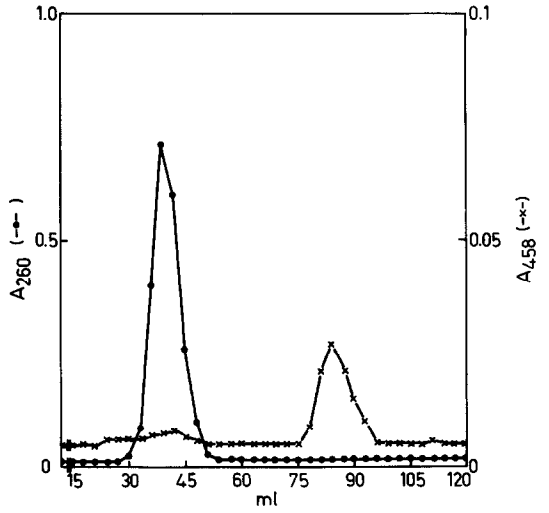

Fig. 5. Effect of EDTA on the ribosome-Aureovocin complex. Washed ribosomes were resuspended in 1 ml 0.01M Tris-HCl buffer pH 7.5 containing 5×10^{-3}M EDTA. The mixture was chromatographed on a Sephadex G-50 column (2 X 50 cm) which was equlibrated with the same buffer. A_{260} (•—•) of r-RNA; A_{458} of Aureovocin (x—x).

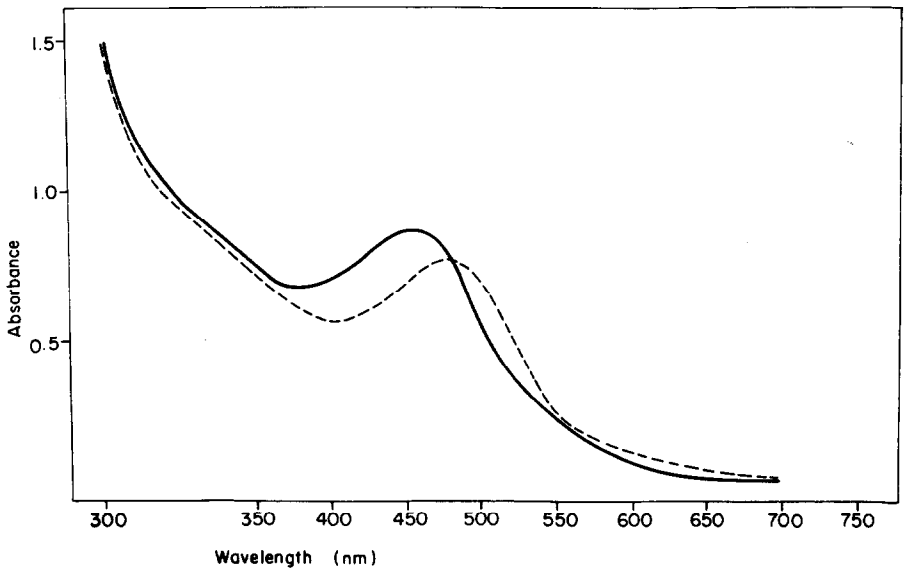

Fig. 6. Visible spectra of the Aureovocin-ribosome complex in buffer I (----) and after addition of 5×10^{-3} M EDTA (——).

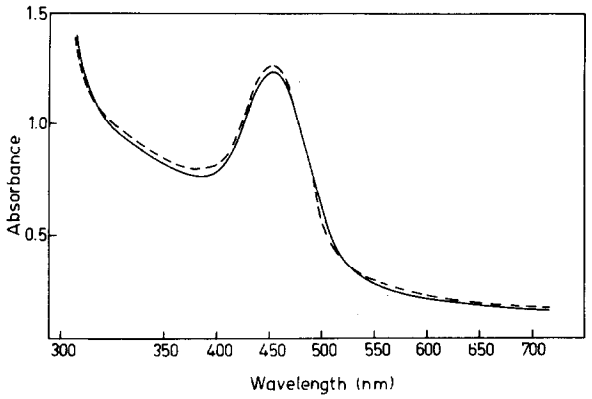

Fig. 7. Visible spectra of Aureovocin in buffer I (— —) and after addition of 5 X 10^{-3}M EDTA (——).

value of the pigment were identical with Aureovocin [7].

Direct evidence for existence of the complex between Aureovocin and ribosomes was obtained by molecular filtration. When the washed ribosomes were resuspended in buffer I and filtred through Sephadex G-50 (fig. 3), the peaks corresponding to ribosomes and the pigment were at the same position. This indicates that the low molecular compound must be tightly bound to the ribosomes. This complex is stable in presence of β-mercaptoethanol and in pH range of 6.5–8.0. However, at the lower pH's, ribosomes were split and the pigment was freed from the complex (fig. 4). Moreover, the Aureovocin can be released from the complex by addition of EDTA as shown in the following experiment. To the ribosome pellet was added EDTA to final concentration of 5 X 10^{-3}M. The mixture was placed in ice for 5 min and applied to a Sephadex G-50 column which was equilibrated with 0.01M Tris-HCl buffer pH 7.5 containing 5 X 10^{-3}M EDTA (buffer II). The results in fig. 5 show that a substantial part of Aureovocin bound by ribosomes was released from the complex. The dissociation of the complex is also accompanied by a shift of the absorption peak (λ_{max} 478) to a shorter wavelength (λ_{max} 458) which is characteristic of the free pigment in the visible region (fig. 6). On the other hand the addition of the same amount of EDTA to a solution of Aureovocin in buffer I was without any effect on the visible spectrum (fig. 7).

These findings strongly support the suggestion that Mg^{2+} is involved in binding of Aureovocin to ribosomes.

References

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