INFLUENCE OF THE 50S RIBOSOMAL SUBUNIT ON THE ABILITY OF THE 30S RIBOSOMAL SUBUNIT FROM Escherichia coli TO BIND DIHYDROSTREPTOMYCIN

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SUMMARY

The binding of dihydrostreptomycin to the 30S ribosomal subunit from Escherichia coli was stimulated by the concomitant addition of 50S ribosomal subunit which has no ability to bind the antibiotic. This result may indicate that association with 50S subunit induces an alteration in the conformation of the 30S subunit and a consequent change in its ability to bind dihydrostreptomycin.

Streptomycin and dihydrostreptomycin (DHSM) interfere with ribosomal function in sensitive bacteria (1-3). It has been shown that these antibiotics bind to the 30S but not to the 50S subunit of ribosomes from E. coli (4). Ozaki et al. (5) showed that a single protein (P_{10}) of the 30S subunit is responsible for both the sensitivity of the ribosomes to streptomycin and the ability of the ribosomes to bind the antibiotic. Further, Vogel et al. (6) suggested that the ability of ribosomes to bind DHSM depends upon their conformation.

During a preliminary investigation of the binding of DHSM, we noticed that 30S ribosomal subunits have much weaker affinity for this antibiotic than 70S ribosomes. This observation suggested that the combination with 50S ribosomal subunits to form 70S ribosomes has an influence on the ability of 30S subunit to bind DHSM.

This communication deals with the effect of the 50S subunit on this binding and the results show that the binding ability of 30S subunit is stimulated by the concomitant addition of 50S subunit.

MATERIALS AND METHODS

The bacterial strains used for this study were E. coli Q13 (streptomycin sensitive) and streptomycin resistant strain, ESO2, as described previously (7). Ribosomes were prepared from each strain and washed with 1M NH_4Cl according to the previous paper (8). The ribosomes were separated into 30S and 50S subunits by overnight dialysis against about 1000 times their volume of 10 mM Tris-HCl (pH 7.8) buffer containing 0.1 mM magnesium acetate, 70 mM KCl and 0.05 mM dithiothreitol, with one change of outer liquid. One ml of the resulting ribosomal solution was layered on 15 ml of a 5-20% linear sucrose gradient and the subunits were fractionated after centrifugation for 16 hr at 18,000 rpm using a Beckman SW 25.3 rotor. The 30S subunit preparations were usually free of detectable contaminating 50S subunits, while some of the 50S subunit preparations contained from 5-10% of 30S subunits.

The reaction mixture used to study the binding of DHSM to ribosomes contained the following components in a final volume of 125 µl; 50 mM Tris-HCl (pH 7.8), 16 mM magnesium acetate, 70 mM KCl, 95,500 cpm of ^{3}H-DHSM (400 µµmoles) and a specified amount of ribosomes. Incubation was carried out at 37°C for 20 min. After incubation, the reaction tube was immediately put into an ice bath and the reaction mixture was diluted with 5 ml of cold buffer containing 10 mM Tris-HCl (pH 7.8), 70 mM KCl and 16 mM magnesium acetate. This diluted mixture was poured onto a Millipore filter (HA, 0.45 µ, prewashed with the same buffer) and the filter was washed 5 times with 3-ml portions of cold buffer. The ribosome bound ^{3}H-DHSM adsorbed on the filter was counted with a Beckman liquid scintillation counter using conventional toluene scintillation solution.

^{3}H-DHSM was obtained from the Radiochemical Centre, Amersham, England.

RESULTS AND DISCUSSION

As expected from previous work (4), ^{3}H-DHSM bound preferencially to 30S

Table 1

Binding of DHSM to 30S, 50S and 70S ribosome preparations

The preparations used in Exp. 1, 2, and 3 were prepared separately. To contain roughly equimolar amounts of ribosome or ribosomal subunits, 1, 2, and 3 A_{260} units of 30S, 50S and 70S preparations from E. coli Q13 were added, respectively. Other conditions were as described under MATERIALS AND METHODS.

Ribosome preparation	^{3}H-DHSM bound (cpm) Exp. 1	Exp. 2	Exp. 3
30S	3,634	2,801	3,092
50S	581	912	1,476
70S	10,877	9,647	11,035

ribosomal subunits (Table 1). The binding observed with 50S ribosomal subunits was possibly due to contamination of the preparations with 30S subunits, though the amounts of ^{3}H-DHSM bound to these preparations seemed to be much larger than expected from the amounts of contaminating 30S subunits. Further, the amount of ^{3}H-DHSM bound to 3 A_{260} units of 70S ribosome preparations was much larger than that attached to one A_{260} unit of 30S subunits. If we assume that 3 A_{260} units of 70S ribosomes are made up of roughly one A_{260} unit of 30S subunits and two A_{260} units of 50S subunits, then the result may indicate that the formation of 70S ribosomes from 30S and 50S ribosomal subunits induces an alteration in the ability of 30S subunits to bind ^{3}H-DHSM. This would also explain the anomalous binding of ^{3}H-DHSM to these 50S subunit preparations.

Strong support for this consideration was obtained by investigation of the effect of the addition of 50S subunits to 30S subunits on the binding of ^{3}H-DHSM. As shown in Fig. 1, the binding of the antibiotic to 30S subunits was remarkably stimulated by the concomitant additions of 50S subunit preparation, which itself does not bind a significant amount of ^{3}H-DHSM. It has been well established that the ribosomes obtained from streptomycin resistant E. coli cells hardly bind DHSM (4, 7). The

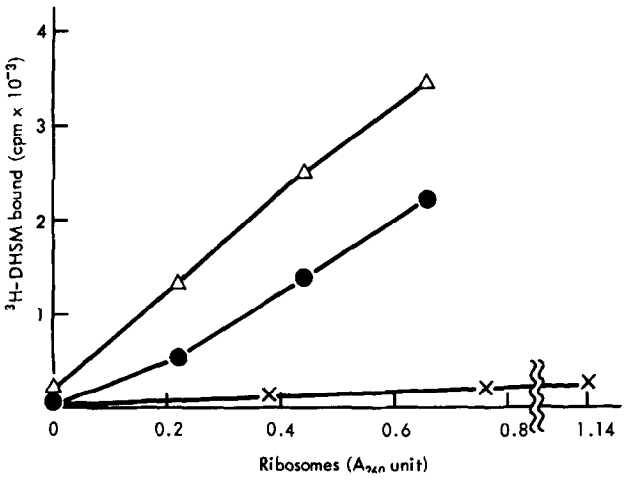

Fig. 1. Effect of 50S ribosomal subunits on the binding of DHSM to 30S ribosomal subunits. The reaction mixture contained the indicated amounts of ribosomal subunit preparations from E. coli Q13. Other conditions were as described under MATERIALS AND METHODS.
●——● 30S subunit preparation; x——x 50S subunit preparation; Δ——Δ 30S subunit preparation with 0.76 A_{260} unit of 50S subunit preparation

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addition of excessive amounts of such 70S ribosomes from streptomycin resistant strain, as shown in Fig. 2, strongly enhanced the binding of ^{3}H-DHSM to 30S ribosomal subunit from streptomycin sensitive strain. Studying the polyphenylalanine synthesis by a mixture of streptomycin resistant 70S ribosomes and streptomycin sensitive 30S ribosomal subunits, Takeda and his colaborators (9) demonstrated the dissociation of 70S ribosomes into 30S and 50S ribosomal subunits and subsequent exchange of the dissociated 30S subunits and externally added 30S subunits in the reassociation of 70S ribosomes in the conditions for polyphenylalanine synthesis. The results shown in Fig. 2, may be explained by assuming that the 70S ribosomes from streptomycin resistant cells dissociated into 30S and 50S ribosomal subunits even in the absence of mRNA and supernatant enzymes, and that some of the dissociated 50S subunits reassociated with externally added streptomycin sensitive 30S subunits, resulting in enhancement of the ability of the 30S ribosomal subunits to bind ^{3}H-DHSM.

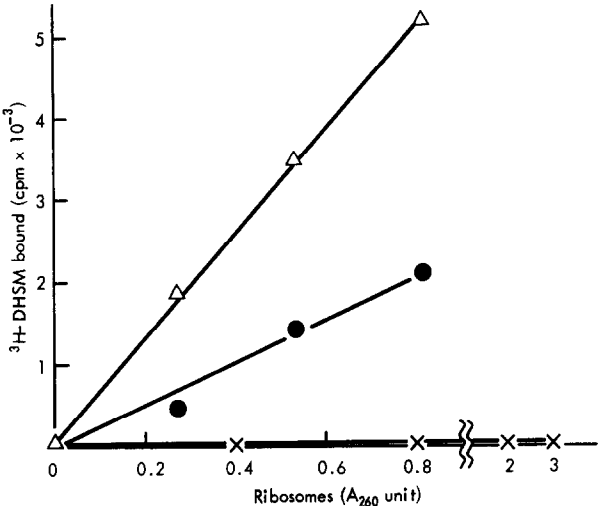

Fig. 2. Effect of 70S ribosomes from streptomycin resistant strain on the binding of DHSM to 30S ribosomal subunits. The reaction mixture contained the indicated amounts of ribosome preparations from streptomycin sensitive strain (Q13) and resistant strain (ESO2). Other conditions were as described under MATERIALS AND METHODS.
●——● 30S subunit preparation from Q13 strain; x——x 70S ribosome preparation from ESO2 strain; Δ——Δ Q13 30S subunit preparation with 3 A_{260} units of ESO2 ribosome preparation

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It has recently been reported that G factor dependent GTPase activity of 50S ribosomal subunits is enhanced by the addition of 30S ribosomal subunits (10, 11). This has led to the consideration that the conformation of 50S ribosomal subunits is altered in the presence of 30S subunits and that this alteration of conformation leads to enhancement of the GTPase activity. The results obtained in this paper may be the simple and clear indication of an alteration of the conformation of 30S ribosomal subunits on combination with 50S subunits with a consequent change in the ability of the 30S subunits to bind DHSM.

From these findings it is very tempting to consider the reasonable working hypothesis that 30S and 50S ribosomal subunits have distinct conformations characterizing each stage in the ribosomal cycle, and that such characteristic conformations are of importance to fulfil a specific role in each step of protein biosynthesis.

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