EFFECT OF DIHYDROSTREPTOMYCIN OF THE BINDING OF SPECIFIC sRNA TO 30S RIBOSOMAL SUBUNITS AND RE-ASSOCIATED 70S RIBOSOMES

Iwao Suzuka

Department of Biophysics
National Institute of Animal Health
Kodaira, Tokyo, Japan

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During the course of studies on the role of ribosomal subunits in the binding of specific sRNA to ribosomes, it was found that the 30S subunit by itself binds phenylalanyl sRNA, and the addition of 50S subunits to a 30S subunit preparation resulted in about a twofold stimulation of phenylalanyl sRNA binding capacity of the original 30S subunit preparation (Kaji et al., 1965, Suzuka et al., 1965, 1966). The binding of phenylalanyl sRNA was dependent on the presence of poly U (polyuridylic acid), indicating its specific nature. The 50S subunit can not bind phenylalanyl sRNA in the presence of poly U.

Streptomycin inhibits the specific binding of phenylalanyl sRNA to 70S ribosomes and 30S subunits (Pestka et al., 1965. Kaji and Kaji, 1965, Kaji et al., 1966). It was therefore of interest to examine the effect of dihydrostreptomycin on the specific binding of phenylalanyl sRNA to re-associated 70S ribosomes. In this communication I report that dihydrostreptomycin causes strong inhibition on the stimulatory effect of 50S subunits in the binding reaction.

The binding of aminoacyl sRNA to 30S subunits and re-associated 70S ribosomes was reported by Pestka & Nirenberg (1966).

Materials and Methods - Preparation of ribosomes, 50S and 30S subunits of ribosomes from E. coli B have been described in the preceding communications

(Suzuka et al., 1965, Kaji et al., 1966). E. coli sRNA and C^{14}-phenylalanyl sRNA were prepared according to Kaji et al., (1965). The binding of phenylalanyl sRNA to ribosomes and their subunits was carried out as described by Nirenberg and Leder (1964). Specific radioactivity of C^{14}-phenylalanine was 200 μc/μmole (counting efficiency, 10^6 cpm/μc). Poly U was purchased from Miles Chemical Laboratory. Dihydrostreptomycin was obtained from Takeda Chemical Industries.

Results and Discussion - In the experiment shown in Fig. 1, the effect of dihydrostreptomycin on the specific binding of phenylalanyl sRNA to 30S subunits and re-associated 70S ribosomes was studied in the presence of poly U.

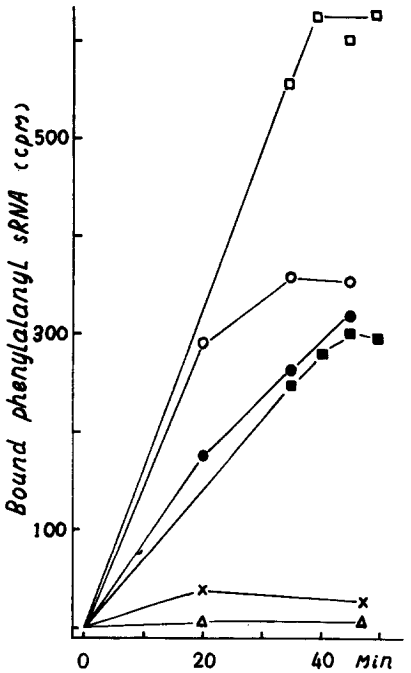

Figure 1. Time course of specific binding of phenylalanyl sRNA to 30S subunits and re-associated 70S ribosomes in the presence or absence of dihydrostreptomycin

Thirty mg of E. coli ribosomes were suspended in 2 ml of solution containing 0.01 M phosphate buffer (pH 7.0) and 0.001 M magnesium acetate. By this procedure 70S ribosomes were dissociated into 30S and 50S subunits (Tissieres et al., 1959). To obtain subunits, this suspension was layered top of 28 ml of a 5-20 % linear sucrose gradient in a buffer which contained 0.06 M KCl, 10^{-4}M magnesium acetate, 0.006 M β-mercaptoethanol and 0.01 M Tris-HCl (pH 7.6). The tube was centrifuged in a Spinco SW-25 rotor for 12 hours at a speed of 20,000 rpm at -2°. The complete reaction mixture for binding of phenylalanyl sRNA contained the following in μ moles/0.6 ml: 30 Tris (pH 7.1), 5.4 β-mercaptoethanol, 15 magnesium acetate, 0.375 puromycin.

In addition, it contained 15,000 cpm of C^{14}-phenylalanyl sRNA, 60 μg of poly U, 20 μg of 30S subunits and 90 μg of 50S subunits. The incubation was carried out at 24°. At various time intervals, 0.14 ml aliquots were taken, then passed through a cellulose nitrate millipore filter (Nirenberg and Leder, 1964) and the bound phenylalanyl sRNA was counted. o——o, 30S subunits; ●——●, 30S subunits and 300 μg of dihydrostreptomycin; □——□, 30S subunits and 50S subunits; ■——■, 30S subunits, 50S subunits and 300 μg of dihydrostreptomycin; △——△, 30S subunits and 50S subunits, but poly U was omitted from the mixture; x——x, 50S subunits.

As shown in this figure, dihydrostreptomycin inhibited about 40% of the binding activity of 30S subunits at 20 min. However, about 90 % of the binding activity of 30S subunits in the absence of dihydrostreptomycin was observed, when the incubation was carried out for 45 min in the presence of dihydrostreptomycin. On the other hand, when 50S subunits were added to the reaction mixture of 30S subunits, the binding activity of the mixtures (re-associated 70S ribosomes) was approximately doubled, confirming the preceding report (Suzuka et al., 1966). When dihydrostreptomycin was present in the mixtures, however, the binding activity of the mixtures was diminished to about 50 % at the various time intervals, suggesting that dihydrostreptomycin abolished almost completely the stimulatory effect due to 50S subunits. In these conditions, dihydrostreptomycin had no influence on the formation of 70S ribosomes from their subunits.

In the experiment shown in Table 1, the effect of dihydrostreptomycin on the stimulatory effect of 50S subunits was studied under conditions where most of the active 30S subunits had phenylalanyl sRNA. In this case also the addition of 50S subunits resulted in approximately twofold increase of the bound phenylalanyl sRNA. On the other hand, when both 50S subunits and dihydrostreptomycin were added at 20 min after the onset of the binding reaction of phenylalanyl sRNA to 30S subunits, the binding activity of the mixtures was inhibited apparently 10-15 % by dihydrostreptomycin. It should be pointed out, however, that there was 60-70 % inhibition of the stimulatory effect of 50S subunits, because the binding activity of the 30S subunit alone was appreciably stimulated when dihydrostreptomycin was added at 20 min and the reaction was allowed to continue for another 25 min. In Fig. 1, the effect of dihydrostreptomycin on the stimulatory effect of 50S subunits was studied under conditions where none of the ribosomes had bound phenylalanyl sRNA. It is noted that under these conditions, dihydrostreptomycin inhibited remarkably the stimulatory effect of the 50S subunit. Therefore, one can explain the present observations that dihydrostreptomycin acts strongly at

Table 1. Effect of 50S subunits and dihydrostreptomycin added after the onset of binding reaction of phenylalanyl sRNA to 30S subunits

	Dihydrost-reptomycin (μg)	Additions 50S subunits (μg)	30S subunits (μg)	Bound phenylalanyl sRNA (cpm)	Inhibition (%)	Effect of 50S subunits (cpm)	Inhibition on the 50S effect (%)
Exp. 1	0	0	7	342*	-	-	-
	0	0	7	355	-	-	-
	0	30(at 20')	7	612	-	257	-
	100(at 20')	30(at 20')	7	521	15	107	58
	100(at 20')	0	7	414	-	-	-
Exp. 2	0	0	5	395*	-	-	-
	0	0	5	406	-	-	-
	0	29(at 20')	5	715	-	309	-
	100(at 20')	29(at 20')	5	638	11	88	72
	100(at 20')	0	5	550	-	-	-

*The incubation was carried out for 20 min.
The complete reaction mixture for binding of phenylalanyl sRNA contained the following in μmoles/0.2 ml: 10 Tris(pH 7.1), 5 magnesium acetate, 1.8 KCl, 0.18 β-mercaptoethanol, 0.125 puromycin. In addition, it contained 5,000 cpm of C^{14}-phenylalanyl sRNA, 20 μg of poly U, 50S subunits, 30S subunits and dihydrostreptomycin as indicated in the table. The binding reaction was carried out for 45 min at 24°. Some components were added at 20 min as indicated in the table after the onset of binding reaction and then the incubation was carried out for another 25 min.

the level of the interaction between messenger RNA and the sRNA which is bound by the addition of 50S subunits.

It is tempting to explain the stimulatory effect of 50S subunits on the assumption that two sRNA molecules are bound to one 70S ribosome particle (Kaji and Kaji, 1963; Arlinghaus et al., 1964, Warner and Rich, 1964). The present observations are consistent with the hypothesis that one molecule of sRNA can bind to the 30S subunit and the second binding site is generated by the formation of 70S ribosomes. In other words, a possible explanation is that a 30S subunit would have two binding sites for the specific sRNA binding and the binding reaction proceeds in two steps; in the first step the one site can bind the first sRNA in the absence of 50S subunits, and in the second step the another site on a 30S subunit is stimulated by the addition of 50S subunits and the binding of the second sRNA takes place. The second step is much more sensitive to dihydrostreptomycin than the first one. In the preceding report (Suzuka et al., 1966), tetracycline was shown to have an inhibitory effect on the first step rather than the second step, since the inhibitory effect of tetracycline was much less when both 50S subunits and tetracycline are added at 20 min after the onset of the binding reaction of phenylalanyl sRNA to 30S subunits and the incubation is carried out for another 20 min, whereas the twofold stimulatory effect of 50S subunits is abolished completely when tetracycline is added at 0 min in the reaction mixture of 50S subunits and 30S subunits. It has been shown that LiCl inhibits the specific binding of sRNA to 70S ribosomes and 30S subunits (Suzuka et al., 1965). LiCl action also would give information on the distinguishable two steps in the binding reaction of sRNA. Experiments are in progress along this line.

Summary - Dihydrostreptomycin inhibited strongly the twofold stimulation of phenylalanyl sRNA bidning which is induced by the addition of 50S subunits on the binding of phenylalanyl sRNA to the 30S subunit-poly U complex.

REFERENCES

Arlinghaus, R., J. Shaeffer, and R. Schweet, Proc. Natl. Acad. Sci. U. S., 51, 1291 (1964)
Kaji, A., and H. Kaji, Biochem. Biophys. Res. Commun., 13, 186(1963)
Kaji, A., H. Kaji, and G. D. Novelli, J. Biol. Chem., 240, 1185(1965)
Kaji, H., and A. Kaji, Proc. Natl. Acad. Sci. U. S., 52, 1541(1964)
Kaji, H., and A. Kaji, Proc. Natl. Acad. Sci. U. S., 54, 213(1965)
Kaji, H., I. Suzuka, and A. Kaji, J. Biol. Chem., 241, 1251(1966)
Nirenberg, M., and P. Leder, Science, 145, 1399(1964)
Pestka, S. R., R. Mashall, and M. Nirenberg, Proc. Natl. Acad. Sci. U. S., 53, 639(1965)
Pestka, S., and M. Nirenberg, J. Mol. Biol., 21, 145(1966)
Suzuka, I., H. Kaji, and A. Kaji, Biochem. Biophys. Res. Commun., 21, 187(1965)
Suzuka, I., H. Kaji, and A. Kaji, Proc. Natl. Acad. Sci. U.S., 55, 1483(1966)
Tissieres, A., J. D. Watson, D. Schlessinger, and B. R. Hollingworth, J. Mol. Biol., 1, 221(1959)
Warner, J. R., and A. Rich, Proc. Natl. Acad. Sci. U. S., 51, 1134(1964)