THE BINDING OF KASUGAMYCIN TO THE *ESCHERICHIA COLI* RIBOSOMES

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The binding of kasugamycin to the *E. coli* ribosomes has been demonstrated by equilibrium dialysis. Kasugamycin binds to the 70S ribosomes in a molar ratio of 1:1. The association constant is approximately 6×10^4 M^{-1}. The antibiotic binds to the 30S subunit as well as to 70S ribosomes, but only slightly to the 50S subunit. The binding of kasugamycin is not significantly affected by the presence of other aminoglycoside antibiotics: streptomycin, kanamycin, or gentamicin. Kasugamycin does not bind to ribosomes derived from a kasugamycin-resistant mutant, *ksgC*.

Kasugamycin, an aminoglycoside antibiotic, is a selective inhibitor of initiation in bacterial protein synthesis[1,2]. It interferes with 30S initiation complex formation, but does not affect the binding of alanyl-tRNA, the second amino acid of coat protein cistron, on f2 phage RNA. It exhibits little effect on peptidyl transferase reaction. Since kasugamycin does not cause misreading, the mechanism of action seems to be different from that of streptomycin and other aminoglycoside antibiotics[3].

For the purpose of elucidating the mechanism of kasugamycin inhibition of bacterial protein synthesis, the binding of ^{3}H-kasugamycin to the *E. coli* ribosomes has been investigated, and the results are presented in this publication.

Materials and Methods

^{3}H-Kasugamycin (1×10^7 cpm/mg) was generously supplied by Dr. HAMAO UMEZAWA, Institute of Microbial Chemistry, Tokyo. It was prepared by the method of WILZBACH[4], and gave a single spot identical to kasugamycin on cellulose thin-layer chromatography (Avicel),[5] and was microbiologically active.

The ribosomes were isolated from *E. coli* K12, strain AB312 (Hfr, *lac*, *thr*, *leu*, *thi*, *strA*, *fus*) and a kasugamycin-resistant mutant, *ksgC*[6,7] in the logarithmic phase of growth, and washed in buffer, containing 1 M NH_4Cl, 10 mM Tris-HCl, pH 7.5, 10 mM $Mg(AcO)_2$, and 6 mM 2-mercaptoethanol. The ribosomal subunits were prepared by the method described in the previous paper[6].

Equilibrium dialysis was carried out using an apparatus for micro-scale experiments which was kindly provided by Dr. BOYD HARDESTY, University of Texas, Austin, Texas. The ribosomes or the subunits, suspended in 0.1 ml of a standard buffer [10 mM Tris-HCl, pH 7.5, 100 mM NH_4Cl, 10 mM $Mg(AcO)_2$, and 6 mM 2-mercaptoethanol], were placed in a chamber; and ^{3}H-kasugamycin in 0.1 ml of the standard buffer was placed in the other chamber. Both chambers were separated by Visking cellulose tubing membranes. The apparatus was kept at 4°C for 24 hours with gentle shaking. This period was determined long enough for equilibration under the conditions employed. The radioactivity of samples of materials in both chambers was de-

termined with a liquid scintillation counter. The difference of radioactivity between the two samples was taken to represent the amount of bound kasugamycin.

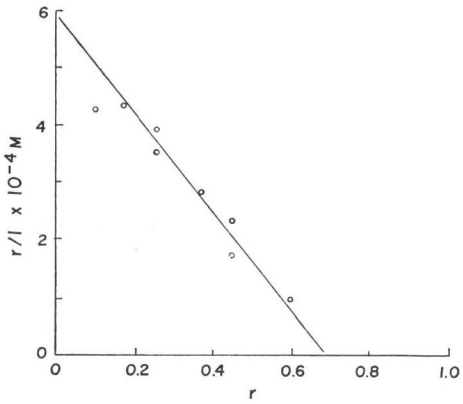
Results

^{3}H-Kasugamycin was observed to bind with the *E. coli* ribosomes. For the purpose of determining the number of binding sites on the ribosome and the association constant of the binding, the equilibrium dialysis was performed over a range of ^{3}H-kasugamycin concentrations. The results were plotted according to the SCATCHARD equation[8] for equilibrium binding, $r/I=Kn-Kr$, where r, I, n and K represent moles of bound ^{3}H-kasugamycin per mole of ribosomes, concentration of free ^{3}H-kasugamycin (M), number of binding sites on the ribosome, and association constant respectively. As illustrated in Fig. 1, the points fall on a straight line. From its intercept on the abscissa the number of the binding sites was 0.7, and from its slope the association constant was estimated to be 6×10^4 M^{-1}.

Fig. 1. SCATCHARD plot for equilibrium binding of ^{3}H-kasugamycin to the ribosomes

r: $\frac{\text{m}\mu\text{moles kasugamycin bound}}{\text{m}\mu\text{mole ribosome}}$

I: molar concentration of free kasugamycin (M)



In order to localize the binding site in the ribosomal subunits, the binding experiments were performed with separated ribosomal subunits. The 30S subunit was found to bind ^{3}H-kasugamycin as well as the 70S ribosomes. In contrast, much less binding of ^{3}H-kasugamycin was demonstrated with the 50S subunit. In the latter case binding might have been due to contamination of the 50S porticles with the 30S subunit (Table 1).

Table 1. Binding of ^{3}H-kasugamycin to the 30S ribosomal subunits.

Ribosomes		^{3}H-Kasugamycin bound
30S particles	5 OD_{260} units	0.041 mμmoles
50S	10	0.013
70S	15	0.076

The procedure of equilibrium dialysis is described in Materials and Methods. One of the chambers contained 2.6 mμmoles of ^{3}H-kasugamycin.

The effects of some aminoglycoside antibiotics on the binding of ^{3}H-kasugamycin to the ribosomes were investigated, and the results are presented in Table 2. Streptomycin, kanamycin, and gentamicin did not substantially affect the binding.

Table 2. Effects of aminoglycoside antibiotics on the binding of ^{3}H-kasugamycin to the ribosomes.

Antibiotics	^{3}H-Kasugamycin bound
None	0.098 mμmoles
Kasugamycin 20 mμmoles	0.000
Streptomycin 20	0.124
Kanamycin 20	0.102
Gentamicin 20	0.086

A chamber contained 15 OD_{260} units of ribosomes and an antibiotic in an amount as indicated. The other chamber contained 2.6 mμmoles of ^{3}H-kasugamycin.

The binding of ^{3}H-kasugamycin was also examined with the ribosomes derived from a kasugamycin-resistant mutant (*ksgC*) *E. coli* AB312 ksg^r. In this organism the resistance has been localized in the 30S ribosomal subunit,

in which protein S2 was found to be altered[6]. ^{3}H-Kasugamycin did not bind significantly to the resistant ribosomes (The data are not shown).

Discussion

In the present study, we have found that there is a single binding site for kasugamycin on the ribosome and that it is localized in the 30S subunit. This finding is in accordance with results that kasugamycin acts on the 30S ribosomal subunit and inhibits the 30S initiation complex formation[1]. The association constant has been found to be 6×10^{4} M^{-1}. The reciprocal of the association constant, 1.7×10^{-5} M, is the concentration of free kasugamycin present when the ribosomes are half-saturated with the antibiotic. It agrees well with the concentration required for 50% inhibition of protein systhesis on the *E. coli* ribosomes with f2 phage RNA[1,2]. The results support a close relationship between the binding of the antibiotic to the ribosomes and the inhibition of protein synthesis.

The binding of kasugamycin has been observed to be reversible (The data are not shown). This is in accordance with the finding that the effect of the antibiotic on the ribosomal functions is reversible[2].

The presence of streptomycin, kanamycin, or gentamicin has little effect on the binding of kasugamycin, suggesting that the binding site of kasugamycin is not related to those of these aminoglycoside antibiotics. This is consistent with the observation that the mechanism of action of kasugamycin is different from that of the other aminoglycoside antibiotics[1-3].

Kasugamycin does not bind significantly to the resistant ribosomes (*ksgC*) in which 30S ribosomal protein S2 is altered[6]. It implies that ribosomal protein S2 may participate in the binding of kasugamycin.

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