QUANTITATIVE BINDING OF ^{14}C-ERYTHROMYCIN A TO *E. COLI* RIBOSOMES

RAFAEL FERNÁNDEZ-MUÑOZ
and DAVID VAZQUEZ

Instituto de Biología Celular,
Velazquez, 144, Madrid 6, Spain

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Studies on the binding of either ^{14}C- or ^{3}H-erythromycin to bacterial ribosomes have shown that the antibiotic specifically binds to the 50S subunit[1~6]. However some workers were unable to study the association constant of the erythromycin-ribosome complex since the SCATCHARD plots for erythromycin binding to ribosomes from either *Bacillus subtilis*[1] or *Escherichia coli*[5,6] deviated from linearity. This work was carried out in order to determine the reasons for this deviation in an attempt to study the dissociation constant of erythromycin binding to *E. coli* ribosomes by using pure preparations of [N-methyl-^{14}C] erythromycin.

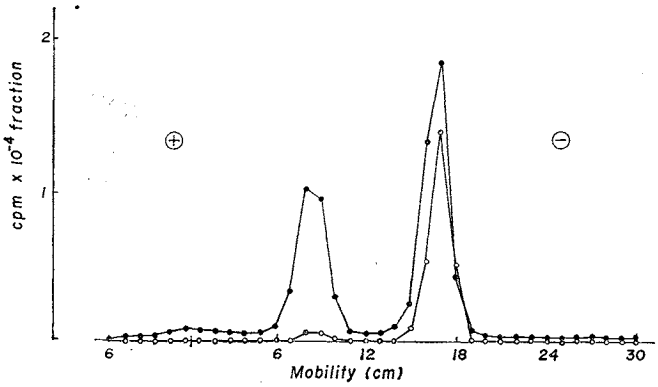
Ribosomes were prepared from log phase *E. coli* MRE 600 by following methods previously described[7]. Estimations of ribosome concentration were carried out on the basis that 1 mg ribosomes/ml has an absorbance of 15 units at 260 nm and that the molecular weight of *E. coli* ribosomes is 2.65×10^{6} [8]. [N-methyl-^{14}C] erythromycin A preparations of specific activities 5.1 μCi/μmol (preparation 1) and 8.3 μCi/μmol (preparation 2) were obtained from Dr. R.E. MCMAHON (The Lilly Research Laboratories, Indianapolis, Indiana, USA) and Dr. J.C.H. MAO (Abbott Laboratories, North Chicago, Illinois, USA). The interaction of [N-methyl-^{14}C] erythromycin A with ribosomes was studied by equilibrium dialysis[5,9] using different concentrations of the antibiotic and ribosomes.

One possible explanation for the anomalous SCATCHARD plots which we have obtained previously in studying ^{14}C-erythromycin binding to *E. coli* ribosomes by either the equilibrium dialysis or ethanol precipitation methods[6] might be the presence of some impurities. For this reason we have analyzed our [N-methyl-^{14}C] erythromycin preparations by electrophoresis at different pH and the electrophoregrams obtained at pH 6.5 are shown in Fig. 1. There is a single peak of radioactivity in the preparation 2. However there are two peaks of radioactivity in the preparation 1 which was the one which we have used previously[5,6]. Peak 1 has the same mobility as preparation 2 of [N-methyl-^{14}C]erythromycin (see Fig. 1). Peak 1 of preparation 1 contains 60 % of the total radioactivity of the preparation and peak 2 represents 40 % of the radioactivity of the preparation.

Studies on ^{14}C-erythromycin (preparation 2) binding to *E. coli* ribosomes have been carried out by the equilibrium dialysis method and the SCATCHARD plot of data obtained is linear as shown in Fig. 2. From this plot the value of $\underline{\nu}$ (binding sites of erythromycin) and K_a (association constant of that binding) could be deduced and were as follows: $\underline{\nu}=1.1\pm0.1$ and $K_a=0.92\times10^{7}\,M^{-1}$. These results confirm previous experimental evidence showing that there is one binding site for

Fig. 1. Profile of radioactivity of preparations 1 and 2 of [N-methyl-^{14}C]erythromycin A.

Electrophoresis was carried out in 10 % pyridine-acetic acid buffer, pH 6.5 for 1 hour at 90 V/cm using Whatman 3 mm paper in a Shandon high voltage apparatus (water cooled). Distance between the cooling plates was 55 cm. Pieces of 1-cm length were cut from the electrophoresis paper (1.5-cm width), mixed in vials with 2.5 ml of a scintillation fluid and radioactivity measured. Profile of radioactivity of preparation 1 (•——•) and preparation 2 (○——○).



erythromycin A per ribosome of *E. coli*[5], *Bacillus subtilis*[1] and *Staphylococcus aureus*[2].

Concerning preparation 1 of ^{14}C-erythromycin we found that peaks 1 and 2 have different specific activity of approximately 4×10^{-3} mCi/mg and 1.3×10^{-2} mCi/mg, respectively. Preliminary binding studies with these peaks have shown that peak 1 (presumably erythromycin A) has an affinity for the ribosome at least twenty times higher than peak 2.

We conclude from the above results that there is one binding site for erythromycin A on *E. coli* ribosomes and that the SCATCHARD plot of the binding is linear with an association constant $K_a=0.92\times10^7$ M^{-1} under our experimental conditions (Fig. 2). Previous results which were obtained presenting an anomalous SCATCHARD plot were probably due to the fact that preparation 1 has more than one component. We do not know the influence that one of the components might have on the binding of the other one.

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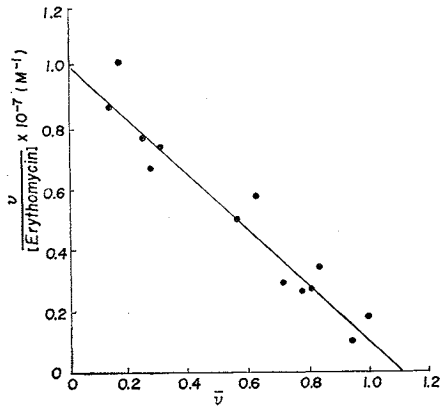

Fig. 2. SCATCHARD plot of data for [N-methyl-^{14}C]erythromycin A binding to *E. coli* ribosomes.

Data are taken from an equilibrium dialysis experiment. Ribosome concentration was 3 mg/ml for tubes with [N-methyl-^{14}C]erythromycin (preparation 2) ranging from 5×10^{-8} M to 8×10^{-7} M and 6 mg/ml for tubes with antibiotic concentrations ranging from 10^{-6} M to 10^{-5} M.

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