DISSOCIATION CONSTANT OF 60S RIBOSOMAL SUBUNIT BINDING TO ENDOPLASMIC RETICULUM MEMBRANES

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SUMMARY:

The apparent dissociation constant for binding of 60S ribosomal subunits to membranes from RER was found to be 2 x 10^{-8} "M" by Scatchard plot analysis. Since the intracellular ribosome concentration is greater than 10^{-6} "M", this suggests that the number of membrane-bound ribosomes is determined by the number of available binding sites. These high affinity binding sites are sufficient to bind 36 µg of total ribosomal RNA per mg of membrane protein, about 30% of values obtained for RER isolated from tissues. SER membranes bind ribosomes with the same affinity as RER, but have one fifth of the number of binding sites.

INTRODUCTION:

Studies of binding of ribosomes to membranes from rough and smooth endoplasmic reticulum (RER and SER) *in vitro* have shown the following (see ref. 1 for review): binding of ribosomes is greatly enhanced if membranes are treated to remove endogenous ribosomes (2-6); membranes from RER bind ribosomes more efficiently than those from SER (2,4,5,7,8); 60S ribosomal subunits bind more efficiently than 40S subunits (6,9); polysomes bind approximately as efficiently as 60S subunits (8,10); ribosomal particles isolated from the free and membrane-bound fractions bind to membranes from RER with equal efficiency (6,8,11,12).

There have not yet been any estimates of the affinity of ribosomes for membranes. Also, differences between RER and SER membranes in binding ribosomes can be explained either in terms of differences in affinity or in the number of ribosome binding sites on the membranes. We have therefore analysed the binding

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of 60S ribosomal subunits to ER membranes using Scatchard plots (13) to determine the apparent dissociation constants and the numbers of sites per unit of membrane.

MATERIALS AND METHODS:

The following have been described previously (6): preparation of RER and SER and of ribosomes labelled with [^{3}H]-orotic acid from livers of starved mice; preparation of ribosomal subunits; removal of endogenous ribosomes from membranes with KCl and puromycin; preparation of cell sap. Protein was measured by the biuret method (14) and ribosome concentration was measured by absorbance at 260 nm; the extinction coefficient determined using the method of Fleck and Munro (15) was 1 absorbance unit at 260 nm equals 45 µg of ribosomal RNA.

Binding of ribosomes to membranes was assayed essentially by the method described previously (6) modified only by using the SW50·1 rotor at 50,000 rpm (274,000 g. max.) to separate membranes from unbound ribosomes.

To study exchange between membrane bound and free 60S subunits, complexes of membranes and labelled 60S subunits were prepared by incubating stripped RER membranes (40 mg of protein), [^{3}H]-labelled 60S subunits (2-3 mg of RNA) and cell sap (50 mg of protein) for 5 min. at 37°C in 12 ml of the same buffer as used for binding assays. The complexes were then separated from unbound subunits by layering 3 ml aliquots over 6 ml of 20% sucrose in buffer and centrifuging 20 min. at 50,000 rpm (275,000 g. max.) in the Beckman Ti 50 rotor. The membranes were then resuspended, incubated with unlabelled 60S subunits or polysomes, and subsequently processed as for the ribosome binding assay.

RESULTS:

Table 1 presents values for the apparent dissociation constant

of the binding of 60S subunits to membranes (K_D) and the µg of ribosomal RNA that bind per mg of membrane protein (n), for membranes of stripped RER and SER. These values were derived from Scatchard plots of data from assays of binding of a range of concentrations of 60S ribosomal subunits to constant amounts of membranes. Typical plots are shown in Fig. 1. The results show that membranes from SER contained approximately one fifth as many ribosome binding sites as membranes from RER, and that the sites on SER and RER had equal affinities for 60S ribosomal subunits.

Since the Scatchard plot is an expression of the law of mass action, we have investigated whether ribosome binding to membranes is reversible. Table 2 shows that only some membrane-bound 60S ribosomal subunits were removed from membranes when complexes were incubated in the presence of excess unlabelled 60S subunits or polysomes. These observations therefore indicate that exchange of membrane bound 60S ribosome subunits with free ribosomal particles does occur, but that it is not a rapid process at least under the conditions of binding. In essence, we confirm the results of Borgese, Blobel and Sabatini (16).

DISCUSSION:

Under the conditions of the binding assays, we were not able to demonstrate full equilibrium between free and membrane-bound ribosomes. Also, both 60S ribosomal subunits and the membrane vesicles are large structures that must be considered to be in suspension rather than in solution. Therefore, since Scatchard plots are derived directly from the law of mass action (13), their use in this context is open to question. However, 60S ribosomal subunits bound to membranes *in vitro* can readily be removed by increasing the concentration of monovalent cations (6), suggesting

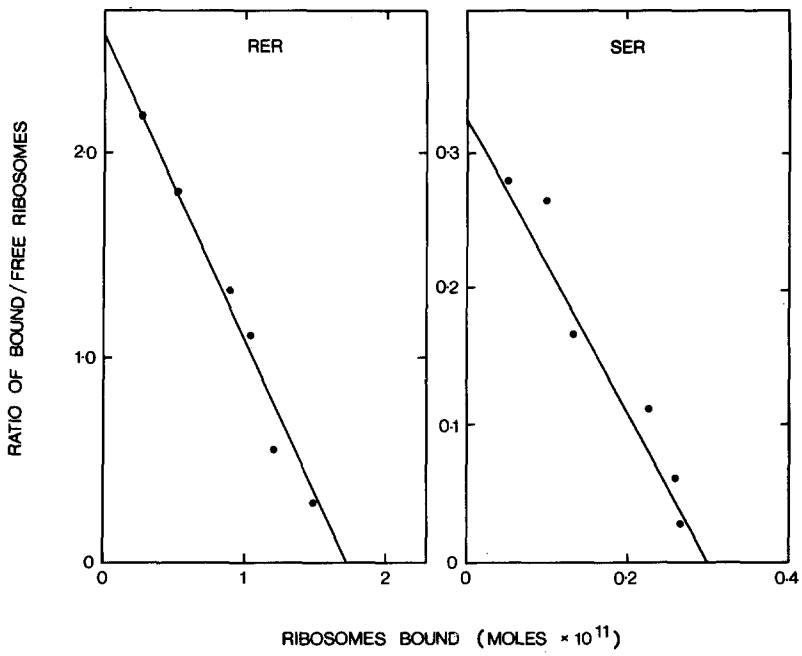

FIGURE 1. Representative Scatchard plots of binding of 60S ribosomal subunits to membranes from RER and SER.

Binding assays contained 1 mg of membrane protein, 60S ribosomal subunits (5-250 µg of RNA) and 100 mM NH_4Cl. Other conditions were as described under Methods.

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that the binding reaction is in fact reversible. Also linear Scatchard plots were obtained (Fig. 1), indicating that the law of mass action does apply. We therefore suggest that analysis of the binding data by Scatchard plots gives useful values for K_D and n.

We have found that the apparent dissociation constant for 60S ribosomal subunit attachment to membranes is approximately 2×10^{-8} "M". Published values for total liver RNA are of the order of 5-10 mg per g wet weight of tissue (19,20). Assuming that 80% of the tissue RNA is ribosomal, and that intracellular water is 60% of the tissue wetweight, liver concentrations of ribosomes are greater than 10^{-6} "M". This suggests that, in

TABLE 1

K_D and n for binding of 60S subunits to membranes

membrane	K_D "M"	n µg of ribosomal RNA/ mg of membrane protein
RER	$1.9 \pm 1 \times 10^{-8}$ (6)*	36 ± 9 (6)**
SER	$3.3 \pm 2.6 \times 10^{-8}$ (3)*	7 ± 6 (3)**

Varying concentrations of ribosomes were incubated with constant amounts of membranes in the ribosome binding assay described in Methods. Values for the apparent dissociation constant (K_D) and for the numbers of binding sites per mg of membrane protein (n) were obtained from Scatchard plots (13) (see Fig. 1). K_D is expressed in molar units ("M") on the basis of a molecular weight of 1.65×10^6 daltons for the RNA of the 60S subunit (17). The number of ribosomes that bind to the membrane sites of affinity K_D is expressed as µg of RNA in 80S ribosome monomers per mg of membrane protein assuming a combined molecular weight of 2.2×10^6 daltons for the RNA of 60S + 40S subunits (17).

Results are expressed as means ± S.D.

*$p>0.2$
**$p<0.001$

intact liver, at least 99% of membrane binding sites for ribosomes are occupied at any one time. This conclusion is consistent with the observation that it is necessary to remove ribosomes from membranes before significant binding of exogenous ribosomes can be observed (2-6). These observations also indicate that the number of ribosomes attached to membranes on these high affinity binding sites is limited by the number of sites in the cell.

The number of ribosome binding sites per mg of membrane protein gives a ratio of RNA to protein for reconstituted RER of 36 µg of RNA/mg of protein. Measured values for freshly isolated RER have, in our hands, been of the order of 120 µg of RNA per mg of protein. The difference between these two values

TABLE 2

Displacement of membrane-bound 60S subunits

by unlabelled ribosomes

Unlabelled ribosomes added		Label remaining on membranes
type	µg of RNA	%
60S subunit	90	96
"	180	92
"	360	79
Polysomes	225	93
"	450	92
"	900	91

Membrane complexes with [^{3}H]-labelled 60S subunits were prepared as in Methods. Samples of the complexes (2 mg of protein containing 52 µg of labelled ribosomal RNA) were incubated with varying amounts of unlabelled 60S ribosome subunits or polysomes and separated from unbound ribosomes using the same conditions as the binding assays (6). Results are expressed as percentages of the amount of labelled ribosomes remaining on membranes after incubation. Complexes incubated without unlabelled ribosomes were set at 100%.

may be because not all ribosomes present on RER obtained from liver are bound to the high affinity sites (see ref. 1), or because ribosome binding sites on RER membranes are masked due to aggregation in the buffers used to assay binding (unpublished observations).

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