SUSCEPTIBILITY OF 55S MITOCHONDRIAL RIBOSOMES TO ANTIBIOTICS INHIBITORY TO PROKARYOTIC RIBOSOMES, LINCOMYCIN, CHLORAMPHENICOL AND PA114A

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SUMMARY

We have used a modified "fragment reaction" to compare the susceptibility of isolated 55S bovine mitochondrial ribosomes, bacterial (E. coli) ribosomes and eukaryotic (bovine microsomal) ribosomes to the antibiotics lincomycin, PA114A and chloramphenicol. These antibiotics inhibit bacterial and 55S mitochondrial ribosomes, but not microsomal ribosomes, supporting the general notion that 55S mitochondrial ribosomes are of the prokaryotic type. However, 55S ribosomes are discriminated from most prokaryotic ribosomes by their response to lincomycin. Unlike ribosomes from bacteria and yeast mitochondria, 55S mitochondrial ribosomes are inhibited only by very high concentrations of lincomycin.

INTRODUCTION

The 55S ribosomes of animal mitochondria comprise a separate class of ribosomes, distinguished from 70S prokaryotic and 80S eukaryotic ribosomes on the basis of major physical-chemical differences (1-4). 55S mitochondrial ribosomes are considered to be of the prokaryotic type, because of their size (2,4), factor exchangeability (5-7) and susceptibility to chloramphenicol (CAP) but not cycloheximide inhibition (8). In view of their unusual composition and physical properties (2), it seems likely that 55S ribosomes would exhibit corresponding functional differences, some of which may be reflected, for example, in altered susceptibility to antibiotics affecting prokaryotic ribosomes.

This possibility was raised earlier by Linnane and coworkers (9-11) even before the distinctive properties of 55S ribosomes were generally known. They found that protein synthesis in mammalian mitochondria, in contrast to yeast mitochondria, was resistant to some antibiotics affecting prokaryotic ribosomes, erythromycin and lincomycin. In this case, the antibiotic resistance of mammalian mitochondria was ascribed to phylogenetic differences in the mitochondrial protein synthetic apparatus (9). An alternative explanation was advanced by Kroon and De Vries (12-14), however, who asserted that the antibiotic resistance

of mammalian mitochondria resides not in altered mitochondrial ribosomes, but in the relative impermeability of the mammalian mitochondrial membrane to these antibiotics. This interpretation, challenged by Linnane's group (11), recently received additional support (7,15).

Clearly, to resolve this controversy, and to determine the antibiotic susceptibility of mammalian mitochondrial ribosomes, studies with isolated 55S ribosomes are required. For this purpose, we undertook a comparative study of ribosome antibiotic sensitivity, using the fragment reaction (16) to measure peptidyl transferase activity of isolated bovine 55S mitochondrial and 80S microsomal ribosomes, and 70S E. coli ribosomes. While these studies were underway, other groups noted that isolated rat 55S mitochondrial ribosomes are inhibited by erythromycin (7,15). In this communication, we report that isolated 55S ribosomes are also sensitive to lincomycin, but moreover, that this inhibition is achieved only with lincomycin concentrations 100-fold higher than those affecting bacterial ribosomes.

MATERIALS AND METHODS

Mitochondrial 55S and microsomal 80S ribosomes were prepared from partially purified bovine liver mitochondria as described (2). N-Ac-[^{3}H]leu-tRNA was prepared (17,18) from E. coli B tRNA for use in a modified "fragment reaction" (16) to measure the peptidyl transferase activity of ribosomes at 25^{o}. Lincomycin was a gift from the Upjohn Co. and PA114A was donated by the Pfizer Co. Chloramphenicol was obtained from Sigma Chemical Co. puromycin from Nutritional Biochemical Co., E. coli stripped tRNA from General Biochemicals and [^{3}H]leu (55 Ci/mmole), from Schwarz Mann.

RESULTS

Mitochondrial 55S ribosomes are readily separated from 80S microsomal ribosomes by centrifugation in sucrose gradients (Figure 1). The small but variable (5 to 15%) contamination of 55S ribosomes by large subribosomal particles of microsomal ribosomes is assessed by analyzing aliquots of pooled 55S ribosomes in sucrose gradients, under dissociating conditions (Figure 2). Under these

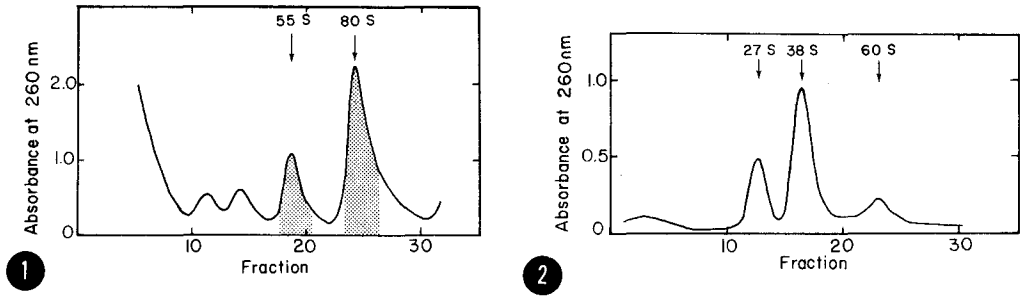

Figure 1. Sucrose density gradient analysis of crude mitochondrial ribosome fraction in buffer T (20 mM Mg^2, 0.1 M KCl, 5 mM 2-mercaptoethanol and 20 mM triethanolamine, pH 7.5). Mitochondrial 55S and microsomal 80S ribosomes were pooled as indicated, and concentrated by centrifugation for use in the peptidyl transferase assay. Centrifugation was for 5 hr at 27,000 rpm in the Beckman SW27 rotor.

Figure 2. Sucrose gradient analysis of the isolated 55S ribosome fraction (see Figure 1) under dissociating conditions in buffer Z (5 mM Mg^{2+}, 0.5 M KCl, 5 mM 2-mercaptoethanol and 20 mM triethanolamine, pH 7.5). Centrifugation was for 13.5 hr at 20,000 rpm in the SW27 rotor.

conditions, the derived mitochondrial subribosomal particles can be distinguished from the residual 60S microsomal subribosomal particles also on the basis of characteristic differences in buoyant density (2).

The peptidyl transferase activity of mitochondrial, microsomal and E. coli ribosomes is compared in Table I. 55S ribosomes show an activity comparable to that of 70S ribosomes but, as noted earlier (19), 80S ribosomes and their 60S subribosomal particles are much less active in this reaction. The specific activity of 60S particles, about 2.5 times that of 80S ribosomes, is only 10-15% that of 55S mitochondrial ribosomes.

The response of these different types of ribosomes to 3 antibiotics known to act on the peptidyl transferase center of prokaryotic ribosomes (21) is seen in Table II. PA114A, CAP and lincomycin inhibit the peptidyl transferase activity of E. coli and mitochondrial 55S ribosomes at concentrations having insignificant effects on 80S ribosomes. Whereas the bacterial and mitochondrial ribosomes respond similarly to the antibiotic PA114A, the 55S ribosomes appear somewhat less sensitive to CAP than do the 70S ribosomes, requiring 10-fold

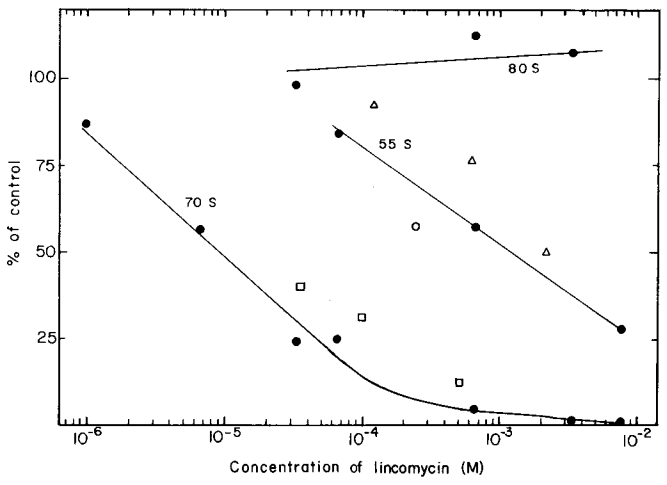

Figure 3. Effect of lincomycin on peptidyl transferase activity of 55S mitochondrial, 70S E. coli, and 80S microsomal ribosomes. The values recorded are averages of 2 to 4 experiments. For comparison, we have also plotted relevant data from other laboratories:

□ inhibition of protein synthesis in intact yeast mitochondria (23).

○ inhibition of protein synthesis in osmotically shocked rat liver mitochondria according to Kroon and De Vries (13).

△ inhibition of protein synthesis in osmotically shocked rat liver mitochondria according to Towers, et al. (11).

higher concentrations of CAP for comparable inhibition. Of considerably greater interest is the failure of low concentrations of lincomycin to inhibit 55S mitochondrial ribosomes, as illustrated in Figure 3. At a concentration of 7×10^{-5} M, lincomycin inhibits the puromycin reaction of 70S ribosomes more than 80% while effecting only marginal inhibition of 55S ribosomes. This finding may account for the relative resistance of protein synthesis in mammalian mitochondria to inhibition by lincomycin (11), irrespective of membrane permeability considerations (12).

The diminished lincomycin sensitivity of these isolated mitochondrial ribosomes is not attributable to their contamination by 60S subribosomal particles of extramitochondrial ribosomes. These subribosomal particles are resistant to lincomycin, but because of their low specific activity in the puromycin reaction (Table I), they contribute a negligible amount of lincomycin-

TABLE I. Peptidyl Transferase Activity of E. Coli Ribosomes and Bovine Mitochondrial and Microsomal Ribosomes

Ribosomes were incubated as described in Methods with puromycin and Ac-[^{3}H]leu-tRNA (10,000 cpm) in a final volume of 0.15 ml. Approximately 1.0 A_{260} unit of 55S or 70S ribosomes, and 10 A_{260} units of 80S ribosomes were used in each assay. Under these assay conditions, 25 to 50% of the Ac-[^{3}H]leu-tRNA is consumed within 15 min. The reactions were terminated by incubation for 3 min at 40° in the presence of 0.6 N KOH (20). 80S microsomal ribosomes recovered from sucrose gradients were dissociated and analyzed by centrifugation in sucrose gradients in buffer Y (5 mM Mg^{2+}, 1.0 M KCl, 5 mM 2-mercaptoethanol, 20 mM triethanolamine, pH 7.5) to obtain 60S subribosomal particles.

	Ac-[^{3}H]leu-puromycin formed (cpm/A_{260}/15 min)		
Ribosome	Expt. 1	Expt. 2	Expt. 3
Cytoplasmic 80S	166	105	----
Cytoplasmic 60S	----	260	----
E. coli 70S	2360	---	5420
Mitochondrial 55S	3480	---	3830

resistant activity to the mitochondrial ribosome fraction. The usual 5-15% of 60S subribosomal particles present in the 55S ribosome fraction thus would contribute no more than 2 or 3% to the total 55S reaction as lincomycin-resistant activity.

Significantly, 50% inhibition of the 55S reaction is achieved only with a lincomycin concentration 100-fold higher than that required for comparable inhibition of the bacterial ribosomes. It should also be noted that 55S bovine mitochondrial ribosomes can be discriminated clearly from 70S bacterial ribosomes, as well as from 80S extramitochondrial ribosomes, by exploiting the differential susceptibility of the ribosomes to lincomycin inhibition in the puromycin reaction. This phenomenon is most apparent at a lincomycin concentration of 1-2 mM. Without detectable effects on 80S ribosomes, this level of the antibiotic virtually abolishes 70S peptidyl transferase activity, while only inhibiting mitochondrial ribosomes about 50%.

DISCUSSION

The puromycin reaction is a convenient assay of ribosome function useful in

ascertaining the susceptibility of isolated 55S mitochondrial ribosomes to some antibiotics of prokaryotic specificity. We have used E. coli ribosomes as a model ribosome of the prokaryotic type in this comparative study. In addition to CAP (16), we find that the antibiotics lincomycin and PA114A inhibit the puromycin reaction with 55S ribosomes. These latter two antibiotics act specifically on probably adjacent or overlapping sites on the large subunit of prokaryotic, but not eukaryotic ribosomes (21). Thus 55S ribosomes appear to be of the prokaryotic type, also by these additional criteria of their susceptibility to lincomycin and PA114A.

Although the responses of 55S and 70S ribosomes to these antibiotics of demonstrated prokaryotic specificity are qualitatively equivalent, we find that these ribosomes exhibit significant quantitative differences in their susceptibility to lincomycin. Our finding that very high concentrations of lincomycin are required to inhibit the puromycin reaction with isolated 55S ribosomes is in accord with the levels required to inhibit protein synthesis in osmotically shocked mitochondria, as reported by Kroon and De Vries (12) and Towers, et. al. (10). The advantage of using isolated mitochondrial ribosomes in studies of antibiotic susceptibility is stressed by the observation that these two laboratories, arguing from similar data (replotted in Fig. 3), should have maintained opposing viewpoints (11,12).

It now appears that the diminished inhibitory action of lincomycin on protein synthesis in mammalian mitochondria derives from two factors. The first of these, a permeability barrier to lincomycin and some other antibiotics at the level of the mitochondrial membrane (11-14) is implicated in the failure of high concentrations of lincomycin to inhibit protein synthesis in intact, undamaged mammalian liver mitochondria (9). It was this phenomenon, paradoxically, which led to the original suggestion of possible phylogenetic differences among mitochondrial ribosomes. The second component of lincomycin resistance resides at the level of the mammalian mitochondrial ribosome, as demonstrated in the present study using isolated bovine mitochondrial ribosomes.

TABLE II. Effect of Chloramphenicol, Lincomycin and PA114A on Peptidyl Transferase Activity of Bacterial, Mitochondrial and Microsomal Ribosomes

Antibiotic	Concentration (mM)	% of Control Activity		
		E. coli 70S	Mitochondrial 55S	Microsomal 80S
Chloramphenicol	0.04	55	67	100
	0.4	14	48	95
	1.0	8	33	96
Lincomycin	0.01	50	--	--
	0.07	18	85	104
	1.0	4	53	111
PA114A	1 μg/ml	49	47	--
	10 μg/ml	22	17	--

Relative to the issue of possible phylogenetic differences in antibiotic susceptibility among different classes of mitochondrial ribosomes, it should be emphasized that pronounced differences do exist in the response to lincomycin of isolated mitochondrial ribosomes from mammals (Fig. 3) and the ascomycete, yeast (23). On the other hand, with erythromycin, another antibiotic implicated in possible phylogenetic differences among mitochondrial ribosomes (9), little difference is noted in the susceptibility of mitochondrial ribosomes from mammals and another ascomycete, Neurospora (24). In view of this observation, and the multiplicity of types of mitochondrial ribosomes (1,25,26) it is premature to generalize about differences in antibiotic susceptibility among mitochondrial ribosomes.

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