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# Differential Antibiotic Sensitivity Determined by the Large Ribosomal Subunit in Thermophilic Archaea

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**Hybrid ribosomes obtained by mixing the ribosomal subunits of the extremely thermophilic archaea *Sulfolobus solfataricus* and *Desulfurococcus mobilis* were tested for their sensitivity to selected antibiotics. It is shown that structural differences in the large ribosomal subunits determine qualitatively and quantitatively the patterns of response to α-sarcin and paromomycin in these species.**

It has been known for some time that the archaea, even members of closely related species, are remarkably heterogeneous in their sensitivity to ribosome-targeted antibiotics (reference 2 and references therein). Since such antibiotics work by interacting with functionally important ribosomal domains, these observations suggest that archaeal ribosomes are structurally diverse, much more so than those of the eubacteria, where the response patterns to protein synthesis inhibitors are usually well conserved.

However, most of the data on the action of ribosome-targeted antibiotics were obtained with the aid of cell-free translation systems (reference 2 and references therein), which cannot always be operated under identical conditions. Thus, the possibility that some of the observed differences (especially quantitative ones) reflected experimental artifacts rather than true ribosome heterogeneity remains.

In this work, we have reconsidered the problem of archaeal ribosome diversity by testing under constant conditions the antibiotic response patterns of hybrid ribosomes, obtained by mixing the ribosomal subunits of the thermophilic, sulfur-dependent archaea *Sulfolobus solfataricus* and *Desulfurococcus mobilis*. These species are closely related phylogenetically but differ both qualitatively and quantitatively in their sensitivity to several protein synthesis inhibitors (1, 9).

We show that *Sulfolobus* and *Desulfurococcus* ribosomes do differ structurally, especially as regards the large subunits. Moreover, we show that the large ribosomal subunit is critically involved in determining qualitatively and quantitatively the response to the small-subunit-targeted inhibitor paromomycin.

**Functional assays with the parent and the hybrid ribosomes.** For a preliminary setting of the experimental system, we assayed the phenylalanine-polymerizing capacities of *D. mobilis* and *S. solfataricus* 70S ribosomes reconstituted from gradient-purified subunits. In vitro translation was tested at 75°C in the presence of 10 mM KCl and 18 mM Mg, by using either *Sulfolobus* or *Desulfurococcus* S-100 fraction as a source of translational factors.

As shown in Table 1, the reconstituted 70S particles from the two species had the same polymerizing activity of about 15 pmol of Phe per pmol of 70S particles in 30 min. However, while *Desulfurococcus* ribosomes were equally active in the presence of either the homologous or the heterologous translational factors, *Sulfolobus* ribosomes accepted only the homologous proteins.

TABLE 1. Phe-polymerizing activity of native and hybrid ribosomes[a]

| Ribosomal subunits (fraction) | cpm (mean ± SD)[b] | pmol of Phe/pmol of 70S particles[c] |
|---|---|---|
| *S. solfataricus* 30S + *S. solfataricus* 50S (*S. solfataricus* S-100) | 21,990 ± 2,430 (3) | 16.3 |
| *S. solfataricus* 30S + *S. solfataricus* 50S (*D. mobilis* S-100) | 2,360 ± 485 (3) | 0.0 |
| *D. mobilis* 30S + *D. mobilis* 50S (*D. mobilis* S-100) | 19,500 ± 2,900 (3) | 14.2 |
| *D. mobilis* 30S + *D. mobilis* 50S (*S. solfataricus* S-100) | 22,360 ± 2,580 (3) | 16.7 |
| *S. solfataricus* 30S + *D. mobilis* 50S (*S. solfataricus* S-100) | 24,700 ± 2,870 (3) | 18.6 |
| *S. solfataricus* 30S + *D. mobilis* 50S (*D. mobilis* S-100) | 9,950 ± 1,050 (2) | 6.3 |
| *D. mobilis* 30S + *S. solfataricus* 50S (*S. solfataricus* S-100) | 20,400 ± 3,530 (3) | 15.0 |
| *D. mobilis* 30S + *S. solfataricus* 50S (*D. mobilis* S-100) | 5,680 ± 1,180 (2) | 2.7 |

[a] Poly(U)-directed cell-free systems were made as previously described (1, 8, 9), and equimolar amounts of the two subunits (6 pmol of each) were added to the mixtures. The samples were incubated at 75°C for 30 min, and hot-trichloroacetic-acid-precipitable radioactivity (expressed as counts per minute) was measured. The specific activity of [$^{14}$C]Phe was about 200 cpm/pmol.

[b] Counts per minute were averaged over several replicate experiments (number given in parentheses). Background values (individual subunits with either S-100 fraction) were 2,380 ± 1,147 cpm (16 replicate experiments).

[c] The amounts (in picomoles) of incorporated phenylalanine were calculated from the average counts per minute after subtraction of background radioactivity.

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Next, we tested the activity of hybrid 70S ribosomes, obtained by mixing *Sulfolobus* and *Desulfurococcus* purified subunits in both possible heterologous combinations. In the presence of *Sulfolobus* S-100 fraction, both hybrid particles displayed the same phenylalanine-polymerizing capacity, comparable to that of the parent 70S ribosomes; with *Desulfurococcus* S-100, however, the hybrid ribosomes were much less active, especially those containing *Sulfolobus* 50S subunits (Table 1).

Such differential behavior of the S-100 proteins, which agrees with a previous report (5), is of unclear significance. For the purpose of the present work, however, all experiments were performed in the presence of *Sulfolobus* proteins, which worked equally well with either type of ribosomes, thus providing a translation system in which the native and the hybrid ribosomal particles had the same protein-synthetic capacity under a fixed set of conditions.

In this situation, any observed difference in antibiotic sensitivity may be safely ascribed to actual ribosome features. Moreover, the use of hybrid particles allows determination of which ribosomal subunit is principally involved in the response to a particular inhibitor.

**Antibiotic sensitivity assays.** For the purpose of this work, we selected the antibiotics paromomycin and α-sarcin, whose binding domains have been well characterized and are located on the small and the large ribosomal subunits, respectively.

**(i) α-Sarcin.** The mechanism of action of the peptide antibiotic α-sarcin has been extensively studied. This drug cleaves the large-ribosomal-subunit RNA at a single site within a highly conserved 14-nucleotide tract located within domain VI at the 3′ extremity of the molecule (3). Eukaryotic ribosomes are much more sensitive than prokaryotic ones to α-sarcin, a fact that has been attributed to the presence of a single nucleotide substitution within the target sequence (15). All archaeal 23S RNAs sequenced so far have the eukaryotic variant of the α-sarcin target sequence; nevertheless, sensitivity to the drug among the archaea is very variable, ranging from high (50% inhibitory dose [$ID_{50}$] ≅ 1 μg/ml) to none (2).

As we showed earlier, the poly(U)-translating activity of *Desulfurococcus* ribosomes is inhibited by α-sarcin at concentrations about 50-fold lower ($ID_{50}$ ≅ 1 μg/ml) than those necessary for a comparable effect on *Sulfolobus* ribosomes ($ID_{50}$ ≅ 50 μg/ml) (1). α-Sarcin sensitivity assays performed with the homologous and heterologous subunit combinations confirmed these findings and clearly showed that the response to this drug is entirely determined by the features of the 50S subunit. As illustrated in Fig. 1, the hybrid subunit combinations had α-sarcin sensitivity profiles essentially overlapping those of the homologous combinations with the same large subunit.

These results indicate that the binding domains for α-sarcin in *Desulfurococcus* and *Sulfolobus* large subunits are indeed structurally different to some extent. Clearly, however, the determinants of α-sarcin sensitivity must include features other than the target 14-nucleotide sequence, as these sequences are identical in the two species (6, 10).

**(ii) Paromomycin.** We have taken into account the ability of paromomycin to provoke decoding errors (4, 13, 14) rather than that of inhibiting translation. As we reported earlier, *Sulfolobus* ribosomes appreciably misread poly(U) in the presence of paromomycin, while *Desulfurococcus* ribosomes do not, even at high concentrations of the drug (9).

Like other mistranslation-inducing aminoglycoside antibiotics, paromomycin affects the ribosomal decoding site, which has been mapped on the small subunit (11); in fact, point mutations in the small-subunit RNA may generate resistance to aminoglycosides. For instance, paromomycin resistance in *Tetrahymena thermophila* (12) and yeast mitochondria (7) has been correlated with the disruption of a CG pair formed between the nucleotides corresponding to positions 1409 and 1491 of the 16S RNA (*Escherichia coli* numbering). However,

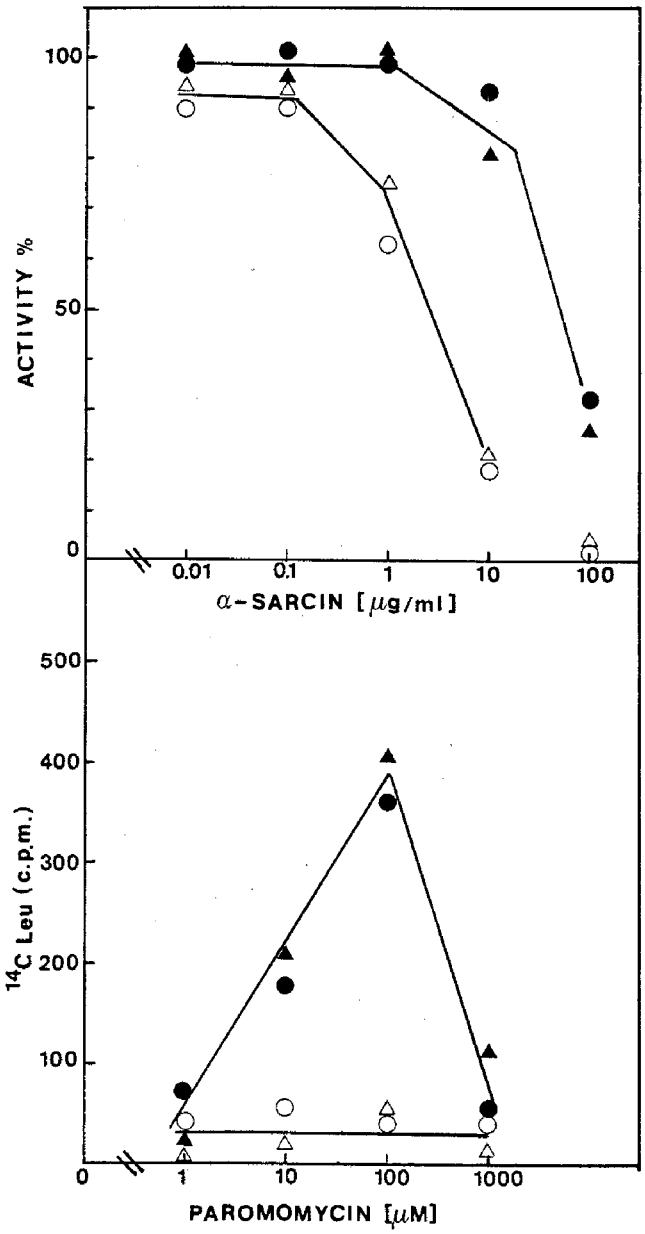


FIG. 1. Antibiotic sensitivity patterns of native and the hybrid ribosomes. (Top) pattern of sensitivity to α-sarcin; (bottom) pattern of paromomycin-induced poly(U) mistranslation. ●, 30S subunit of *S. solfataricus* plus 50S subunit of *S. solfataricus*; ○, 30S subunit of *D. mobilis* plus 50S subunit of *D. mobilis*; ▲, 30S subunit of *D. mobilis* plus 50S subunit of *S. solfataricus*; △, 30S subunit of *S. solfataricus* plus 50S subunit of *D. mobilis*. α-Sarcin was added to poly(U)-primed cell-free systems at the concentrations indicated. Results are expressed as percent activity relative to that of control samples with no antibiotic. The activity of control samples was as shown in Table 1. Paromomycin-induced mistranslation was monitored by measuring the incorporation of [$^{14}$C]leucine into hot-trichloroacetic-acid-precipitable material as described elsewhere (9). In all cases, control experiments were performed with samples containing each of the isolated subunits in turn to ensure that the results observed were not due to contamination with the missing homologous subunit.

all of the archaea so far investigated, including *Sulfolobus* and *Desulfurococcus* spp., have a stable position 1409-1491-equivalent CG pair in their 16S rRNAs (2), thus indicating that, in the sensitive species, some feature other than this critical base pair must be involved in determining sensitivity to the drug.

Paromomycin-induced poly(U) mistranslation was first assayed on 70S ribosomes obtained by recombining the homologous purified subunits of *Sulfolobus* and *Desulfurococcus* spp. (Fig. 1). The results were in very good agreement with those previously obtained with the native 70S particles (9); namely, *Sulfolobus* ribosomes mistranslated poly(U) in the presence of paromomycin with maximal efficiency at a $10^{-5}$ M drug concentration, while *Desulfurococcus* ribosomes were entirely insensitive to the antibiotic.

Paromomycin sensitivity assays with the hybrid ribosomes revealed that only one of the two subunit combinations misread poly(U) in the presence of the drug. Consistently, and unexpectedly, this was the one including *Sulfolobus* 50S and *Desulfurococcus* 30S particles. The dose-response curve of the hybrid ribosomes was exactly superimposable on that obtained with *Sulfolobus* 70S particles, indicating that the *Sulfolobus* 50S subunit determined qualitatively and quantitatively the pattern of sensitivity to paromomycin (Fig. 1).

Thus, the results show that no relevant differences (as to paromomycin accessibility) exist between the decoding domains of *Sulfolobus* and *Desulfurococcus* small ribosomal subunits. Instead, some (yet to be defined) feature of the large subunit is again critically involved in determining the differential antibiotic sensitivities of the two archaeal species.

A rather unexpected corollary of these results is that the large subunit plays an essential role in modulating the interaction of paromomycin with the ribosome. Therefore, the presence of certain nucleotides in the decoding domain of the small-subunit RNA is per se not sufficient to predict sensitivity to paromomycin.

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