Sensitivity of thermoacidophilic archaebacteria to α-sarcin

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The protein synthesis machinery of *Sulfolobus solfataricus*, a thermoacidophilic archaebacterium, is insensitive to most of the known antibiotics that interfere with elongation. α-Sarcin, a cytotoxic protein, inhibits protein synthesis on eukaryotic systems by cleaving a specific sequence of the large rRNA. α-Sarcin is capable of inhibiting protein synthesis on *S. solfataricus* producing a fragment under conditions similar to those which produce it in yeast ribosomes. This result suggests the presence on *S. solfataricus* of the sequence necessary for the enzymatic hydrolysis promoted by α-sarcin.

Thermoacidophilic archaebacteria *Protein synthesis inhibitor* *α-Sarcin* *Evolution*

1. INTRODUCTION

Archaebacteria have been defined as a 'primary kingdom' different from prokaryotes and eukaryotes, based on ribosomal RNA sequences and lipid composition [1]. Protein synthesis inhibitors are considered good phylogenetic markers as they allow a classification of ribosomes based on their sensitivity to different antibiotics [2]. Some reports suggest that archaebacterial ribosomes, while insensitive to typical prokaryotic inhibitors, are sensitive to some eukaryotic antibiotics [3–9]. A recent study of the sensitivity of the protein synthesis machinery of a thermoacidophilic archaebacterium, *Sulfolobus solfataricus*, to different antibiotics, showed an unusual pattern of insensitivity which cannot be attributed to the thermophilic conditions of the assay. Only α-sarcin, a cytotoxic protein, is able to suppress protein synthesis at rather low concentrations, suggesting the presence of the sensitive cleavage sequence detected in eukaryotic, eubacterial and organelle ribosomes [10]. The possible evolutionary implications of this finding are discussed.

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2. MATERIALS AND METHODS

Ribosomes were isolated and poly(U)-directed protein synthesis assayed as in [11] for *Saccharomyces cerevisiae*, as in [12] for *Escherichia coli*, and as in [7] for *S. solfataricus*.

α-Sarcin incubations and fragment identification: The various ribosome preparations were resuspended at a final concentration of 200 A_{260} units/ml in the same reaction mixture used for protein synthesis, without S100, to allow the antibiotic to act under the same conditions in which protein synthesis inhibition is produced. Treatment of the ribosomes with α-sarcin was done at a protein/ribosome ratio of 1:1, for 20 min at 75°C. RNA was extracted and analyzed by gel electrophoresis on polyacrylamide as in [12].

3. RESULTS AND DISCUSSION

An extensive survey of *S. solfataricus* protein synthesis machinery sensitivity to different elongation inhibitors exhibits a rather unique pattern of insensitivity to antibiotics, suggesting a peculiar ribosomal structure for this type of archaebacteria (P. Cammarano et al., submitted). Of the 60 different antibiotics tested only α-sarcin showed significant inhibition of protein synthesis at rather

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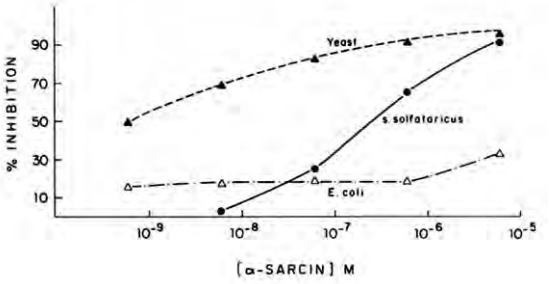

Fig.1. Inhibition of poly(U)-directed polyphenylalanine synthesis by α-sarcin in *Sulfolobus solfataricus, Sacc. cerevisiae* and *E. coli*. Protein synthesis was performed as described in section 2, at 75, 37 and 30°C for *S. solfataricus, E. coli* and *Sacc. cerevisiae*, respectively, for 30 min. The ribosomal concentration was 0.2 μM in all cases. Protein synthesis efficiency ranged from 20 to 40 pmol phenylalanine polymerized per pmol ribosome.

low concentration of the toxin (fig.1). Appropriate controls show that *Sulfolobus* ribosomes are less sensitive than eukaryotic ribosomes, but definitely more sensitive than eubacterial ribosomes, which in our conditions are practically insensitive at 10^{-5} M α-sarcin. The 50% inhibition is obtained at an equimolar concentration of toxin and *S. solfataricus* ribosomes. This mild sensitivity is not due to partial inactivation of the protein under the thermophilic conditions of protein synthesis (75°C), because preincubation of α-sarcin at 75°C for 40 min does not affect its activity when assayed on the yeast system at 30°C (not shown). In contrast, the two toxins mitogillin and restrictocin, which are closely related to α-sarcin, are completely ineffective on *S. solfataricus* while being as effective as α-sarcin when assayed in the yeast system.

When the rRNA extracted from *Sulfolobus* ribosomes treated with α-sarcin in the conditions that produce protein synthesis inhibition is analyzed using polyacrylamide gel electrophoresis a very distinct band appears in the region of low-M_r RNA, which is not present when ribosomes are incubated in the same conditions without α-sarcin (fig.2). This band is only produced when 50 S and 70 S ribosomes are incubated with the toxin, suggesting that the fragment is a product of the large rRNA as in eukaryotic ribosomes. The estimated size of the fragment is around 260 nucleotides.

The lack of sequence of the 23 S rRNA from *Sulfolobus* or any other archaebacteria makes it

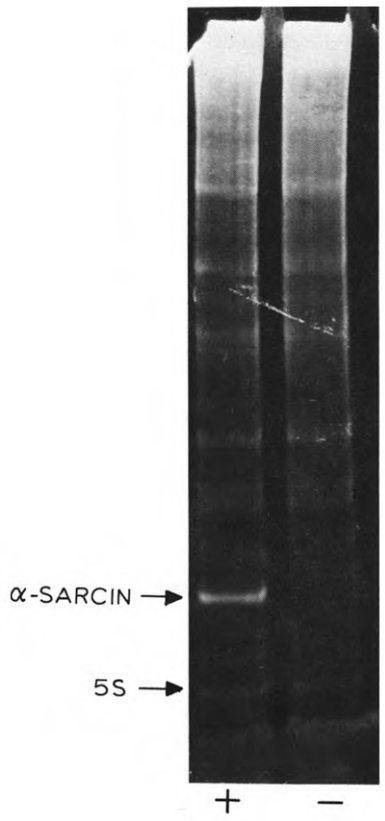

Fig.2. Analysis by gel filtration on polyacrylamide of rRNA from α-sarcin-treated ribosomes. *Sulfolobus solfataricus* ribosomes were incubated under the conditions described in section 2 with (+) or without (−) α-sarcin. RNA was extracted and separated by electrophoresis on polyacrylamide as in [12].

impossible to determine if these organisms contain the consensus sequence recognized by α-sarcin in eukaryotes and eubacteria (fig.3) [10,13], but the inhibition produced by the toxin in *Sulfolobus* and the production of the fragment strongly suggest that the sequence must be present on the large ribosomal RNA. The difference between the activity of α-sarcin on eukaryotic and eubacterial systems is assumed to be due to a simple base change in the eubacterial sequence [13]. At present we cannot give a rational explanation for the mild sensitivity of *S. solfataricus* ribosomes to α-sarcin, but it may possibly be due to: (i) some difference on the consensus sequence, or (ii) a less exposed sensitive site. We should keep in mind that this thermophilic archaebacterium grows at 95°C, so it is reasonable to assume that it needs a much more

SPECIES	CONSENSUS SEQUENCE	TYPE OF rRNA
Rat, X. laevis, Yeast	A G U A C G A G↓A G G A A C	eukaryotic
E. coli, Z. mays chloroplast	A G U A C G A G A G G A C C	eubacterial/organel
Yeast mitochondria	A G U A C G C A A G G A C C	organelle
Mouse mitochondria	A G U A C G A - A A G G A C	organelle
Human mitochondria	U G U A C G A - A A G G A C	organelle

Fig.3. Consensus sequence of the site of cleavage of large rRNA by α-sarcin in different species as proposed in [10,13]. The nucleotide sequences at the cleavage site produced by α-sarcin on large ribosomal RNAs from rat liver, yeast and *E. coli* [14] are compared with analogous regions in other rRNAs [10,13]. Underlined nucleotides denote conservation when compared to the yeast or rat liver sensitive sequence. (↓) designates the cleavage site of α-sarcin.

stable structure to avoid melting of the particle in growth conditions. We have to wait until the site of cleavage is sequenced on *Sulfolobus* to answer this question.

These results support the idea that the unique α-sarcin cleavage site found in eukaryotes, eubacteria and organelle ribosomes is also present in archaebacteria. Preliminary experiments done in collaboration with A. Böck's group in Munich on methanogens and with P. Cammarano's group in Rome on *Thermoplasma* show a similar degree of sensitivity of these groups of archaebacteria to α-sarcin. The comparison of the sequences of these sensitive sites might provide a useful tool with which to analyze the evolution of an important ribosomal site. The specific cleavage of the large rRNA by α-sarcin is able to impair the function of eukaryotic and archaebacterial ribosomes probably disrupting the structure of the particle in such a way that EF-1-catalyzed binding of aminoacyl-tRNA cannot take place [15,16].

So far we cannot explain the lack of activity of restrictocin and mitogillin on the *S. solfataricus* system. These structurally related toxins are fully active on the yeast system. Sequence comparison between α-sarcin and restrictocin shows 86% homology (E. Mendez, submitted) and the site of cleavage is identical to that of α-sarcin (L. Fando et al., unpublished). Some difference in the structure of both proteins must be responsible for this difference in activity, thus supporting the idea that these related toxins are very precise RNases capable of recognizing very specific ribonucleoprotein structures [13].

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