

The ribosomes of *Aspergillus giganteus* are sensitive to the cytotoxic action of α -sarcin

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Ribosomes in lysates prepared from the mycelia of *Aspergillus giganteus* MDH 18894, which are actively secreting α -sarcin, do not contain the α -sarcin lesion. However, the addition of exogenous α -sarcin to these same lysates results in cleavage of the 26 S rRNA of the 60 S ribosomal subunit, characteristic of the cytotoxic action of α -sarcin. We conclude that *A. giganteus* ribosomes are not inherently resistant to the action of α -sarcin but are protected in vivo by producing α -sarcin in an inactive form and/or by the efficient cotranslational secretion of the toxin.

α -Sarcin; Ribosome-inactivating protein; Ribosome inactivation; (*Aspergillus giganteus*)

1. INTRODUCTION

The mold *Aspergillus giganteus* MDH 18894 produces and secretes the toxin α -sarcin [1,2], which inhibits protein synthesis. The cytotoxic action of α -sarcin results from the inactivation of the ribosomes [3,4] brought about by the enzymatic hydrolysis of a specific phosphodiester bond near the 3'-terminus of the largest rRNA [5–7]. The sequence of the α -sarcin cleavage site appears to be universally conserved and has been found in the large rRNA of cytoplasmic and organellar ribosomes of eucaryotes, and in the ribosomes of procaryotes [7]. All ribosomes thus far tested have been found to be sensitive to the hydrolytic action of α -sarcin; i.e. *Saccharomyces cerevisiae* [5,6], wheat germ [5], rat liver [6,7], rabbit reticulocytes [4], and *Escherichia coli* [5,6]. These observations raise the question of how *A. giganteus* protects itself from the cytotoxic effects of α -sarcin. Here, we have sought to determine if the ribosomes of *A.*

giganteus MDH 18894 are inherently resistant to α -sarcin.

2. MATERIALS AND METHODS

The lyophilized culture of *A. giganteus* MDH 18894, which was used in the initial isolation of α -sarcin [2], was a generous gift from T. Watson (Michigan Department of Health). *A. giganteus* cultures were grown at 30°C for 48 h in the α -sarcin inducing medium (medium A) recommended by Olson et al. [1]. *S. cerevisiae* SSL204 [8] was a gift from D.M. Livingston (University of Minnesota) and was grown at 30°C in YM-1 medium [9]. *Aspergillus nidulans* ATCC 10074 was purchased from the American Type Culture Collection and was grown under the conditions recommended by the supplier. Ricin A-chain was purchased from Calbiochem and α -sarcin was a gift from I.G. Wool (University of Chicago).

Ribosomal lysates were prepared by the method of Hofbauer et al. [10] from washed mycelia or cells. The method of Endo and Wool [6] was employed to assay ribosome sensitivity and α -sarcin activity in the culture filtrates. Lysates containing 45 μ g ribosomes were treated with either α -sarcin (100 ng), ricin A-chain (100 ng), or aspergillus culture filtrates (1.0 μ l) for 30 min at 37°C in a total volume of 60 μ l. The reaction was terminated by the addition of 5 vols of 1% SDS in 50 mM Tris-HCl (pH 7.4). Total rRNA was phenol extracted, ethanol precipitated, and analyzed by electrophoresis on 6% polyacrylamide-7 M urea gels [11]. The rRNA was visualized by silver staining [12]. For ricin A-chain treatment, the precipitated rRNA was incubated in 1.0 M aniline-acetate (pH 4.5) for 10 min at 0°C to catalyze the hydrolysis of the rRNA

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at the apurinic site created by ricin [13]. Aniline was removed by ether extraction and the rRNA was ethanol precipitated prior to electrophoresis.

3. RESULTS

The characteristic rRNA lesion produced by α -sarcin can be readily visualized by polyacrylamide gel electrophoresis of total rRNA (fig.1). Since the location of the α -sarcin cleavage site in *A. giganteus* rRNA is unknown, several control experiments were included to locate this site. The fragments produced by the treatment of *S. cerevisiae* lysates with α -sarcin and ricin A-chain followed by aniline hydrolysis were used for comparative purposes. In yeast ribosomes, the α -sarcin cleavage site is near the 3'-terminus of the 26 S rRNA and the resulting fragment (fig.1A, lane 4) is 367 bases in length [6]. Ricin A-chain removes a base one residue to the 5'-side of the α -sarcin cleavage site [13]. The rRNA fragment produced by aniline-catalyzed hydrolysis of the phosphodiester bond at the apurinic site created by ricin treatment (fig.1A, lane 3) is one residue longer than that produced by α -sarcin (Stirpe et al., submitted and [13]). Since the ribosomes of aspergilli are similar to those of yeast with respect to the size of the large rRNA [14], it is likely that the fragments produced by α -sarcin and ricin followed by aniline hydrolysis would also be similar. *A. nidulans* ATCC 10074 does not produce α -sarcin (not shown) and control experiments using lysates of this organism are also shown (fig.1C). The fragments produced by aniline hydrolysis of the rRNA from ricin-modified ribosomes (fig.1C, lane 3) and by α -sarcin (fig.1C, lane 4) in *A. nidulans* ribosomes are indistinguishable from those of yeast rRNA.

We have confirmed the production and secretion of α -sarcin by *A. giganteus* MDH 18894 [1] under the growth conditions we have employed in the preparation of the ribosomal lysates (not shown). This is important since not all isolates of *A. giganteus* produce α -sarcin (Watson, T., personal communication). The rRNA of these ribosomes did not contain the characteristic α -sarcin lesion (fig.1B, lane 1). This demonstrates that the ribosomes have not been acted upon by α -sarcin either in the cytoplasm of the intact cell or during the preparation of lysates. Treatment of these lysates with exogenous α -sarcin, however, caused

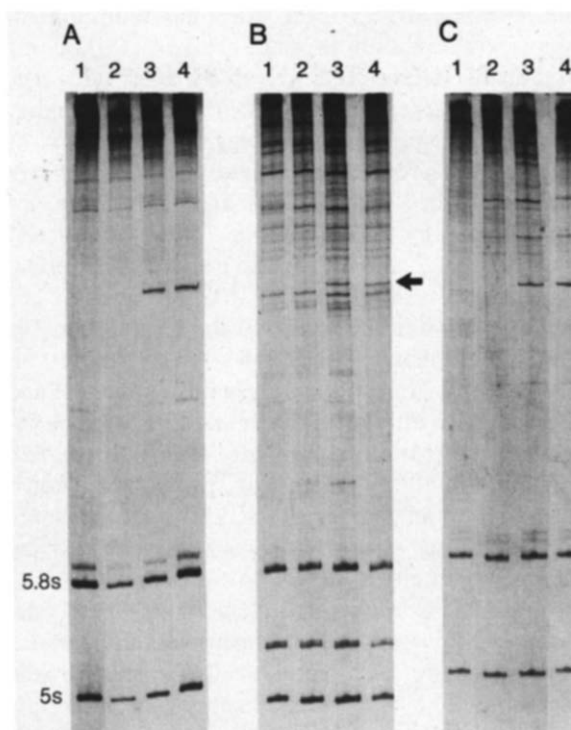


Fig.1. Gel electrophoretic analysis of ribosome sensitivity to α -sarcin and ricin A-chain. Lysates were isolated from: (A) *S. cerevisiae* SSL204; (B) α -sarcin-producing mycelia of *A. giganteus* MDH 18894; and (C) *A. nidulans* ATCC 10074. Lysates were treated as follows: (lanes) 1, control; 2, control followed by aniline treatment of the rRNA; 3, ricin A-chain followed by aniline treatment of the rRNA; 4, α -sarcin. The arrow designates the position of the rRNA fragment produced by α -sarcin.

cleavage of the rRNA (fig.1B, lane 4) as demonstrated by the appearance of the characteristic rRNA fragment (shown by the arrow). The fragment produced by α -sarcin comigrates with the fragment produced by aniline hydrolysis of rRNA from *A. giganteus* ribosomes pretreated with ricin A-chain (fig.1B, lane 3). These observations demonstrate that the ribosomes of *A. giganteus* MDH 18894, which are actively producing α -sarcin, do not contain the α -sarcin lesion in vivo but are themselves inherently sensitive to the action of the toxin.

4. DISCUSSION

A great deal is known about the strategies employed by bacteria to protect themselves from

the cytotoxic effects of the ribosome-inhibiting antibiotics which they produce (review [15]). Some organisms defend themselves by modifying the ribosomal target of the antibiotic. A particularly relevant example of this strategy is provided by the thiostrepton-producing organism *Streptomyces azureus*. The binding to and inhibition of ribosomes by thiostrepton are completely prevented in *S. azureus* by the post-transcriptional methylation of specific bases within the thiostrepton-binding region of the 23 S rRNA. On the other hand, selection for thiostrepton resistance in nonproducing organisms has yielded mutants with alterations in the r-protein which interacts with the portion of rRNA to which thiostrepton binds [15]. Thus, protection against the action of antibiotics which inhibit the ribosome by interacting with rRNA can be achieved by alterations in either rRNA or r-proteins.

Much less is known about the strategies of self-defense employed by organisms which produce toxic proteins that catalytically inactivate the ribosome. The best studied example of such self-defense against a catalytic inhibitor is found in colicin E3-producing bacteria. The cytotoxic effect of colicin E3, like α -sarcin, results from the specific cleavage of rRNA [16]. Ribosomes of the colicin-producing organism are inherently sensitive to the action of colicin E3 but are protected by an 'immunity protein' which binds to and inactivates colicin E3 within the cell [16]. It is also possible that protection against toxic proteins may be achieved through their initial production as inactive zymogens which are activated only after secretion, as exemplified by the zymogens of the pancreatic proteases [17]. Alternatively, protection against the action of extracellular protein toxins may be achieved by efficient co-translational secretion [18]. It is widely assumed that plant ribosomes are resistant to the action of the ribosome-inactivating proteins, such as ricin, which they produce [19]. However, the basis of this presumed resistance has not been defined and it has been suggested that resistance may not apply to all plants which produce ribosome-inactivating proteins [20].

We have demonstrated that ribosomes isolated from α -sarcin-producing cultures of *A. giganteus* do not contain the α -sarcin lesion but that these ribosomes are inherently sensitive to the toxin in vitro. We conclude that *A. giganteus* ribosomes

are protected from the action of the toxin in vivo either because the intracellular form of α -sarcin is inactive and/or because the protein is efficiently secreted.

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