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The site of action of the A-chain of mistletoe lectin I on eukaryotic ribosomes

The RNA *N*-glycosidase activity of the protein

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The site of action of the A-chain of mistletoe lectin (ML-A) from *Viscum album* on eukaryotic ribosomes was studied. Treatment of rat liver ribosomes with ML-A, followed by treatment of the isolated rRNA with aniline, caused the release of a fragment with about 450 nucleotides from 28 S rRNA. Further analysis of nucleotide sequences of this fragment revealed that the aniline-sensitive site of phosphodiester bond was between positions A-4324 and G-4325 in 28 S rRNA. These results indicate that ML-A inactivates the ribosomes by cleaving a *N*-glycosidic bond at A-4324 of 28 S rRNA in the ribosomes as ricin A-chain does.

Mistletoe lectin I; RNA *N*-glycosidase; Ribosomal action site

1. INTRODUCTION

The cytotoxic proteins that inactivate eukaryotic ribosomes include α-sarcin, which is produced by a fungus [1,2]; ricin, abrin and modeccin which are derived from plants [3]; and Shiga toxin which is formed by a bacterium [4,5]. These toxins catalytically inactivate 60 S ribosomal subunits and in this way inhibit peptide chain elongation. α-Sarcin is a novel ribonuclease that hydrolyzes the phosphodiester bond on the 3′-side of the guanosine at position 4325 in 28 S rRNA [6,7]. Ricin A-chain and a group of related proteins, abrin, modeccin, Shiga toxin and VT2-toxin, are also unusual enzymes; they are RNA *N*-glycosidases [8–10]. This group of toxins inactivates 60 S ribosomal subunits by hydrolyzing a *N*-glycosidic bond, i.e. by depurinating the adenosine residue (position 4324 in 28 S rRNA) adjacent to the guanosine that α-sarcin attacks [6–10].

Recently we have purified and characterized another cytotoxic protein, mistletoe lectin, from *Viscum album* [11]. This protein is composed of two subunits, A- and B-chain linked together with an S-S bond. The A-chain inhibits protein synthesis at the ribosomal level. However the molecular mechanism of its action has not yet been elucidated. In this study, we have found that ML-A inactivates the 60 S ribosomal subunits by the same mechanism as ricin A-chain.

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2. METHODS

The following procedures were either described or cited previously [6–9]: preparation of rat liver ribosomes, incubation of the ribosomes with the A-chain of ML toxin, extraction of rRNA with phenol and dodecyl sulfate, aniline-induced chain scission at the modified site in 28 S rRNA, analyses of the nucleic acids by polyacrylamide gel electrophoresis, preparation of 5′-^{32}P-labeled fragment and the method used for sequencing of RNA.

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3. RESULTS AND DISCUSSION

To test if ML-A inactivates rat liver ribosomes by cleaving any phosphodiester bonds of rRNAs in ribosomes, the ribosomes were incubated with catalytic amounts of protein and the extracted rRNA was analyzed by composite gel electroophoresis (fig.1). As can be seen in lanes 2 and 3, this protein has practically no endonuclease activity despite the fact that the ribosomes had less than 10% activity for polyphenylalanine synthesis (not shown). However, when the RNA was treated with aniline at acidic pH before loading onto the gel, a new band which was about 450 nucleotides was produced (lane 5, arrow) from the 28 S rRNA.

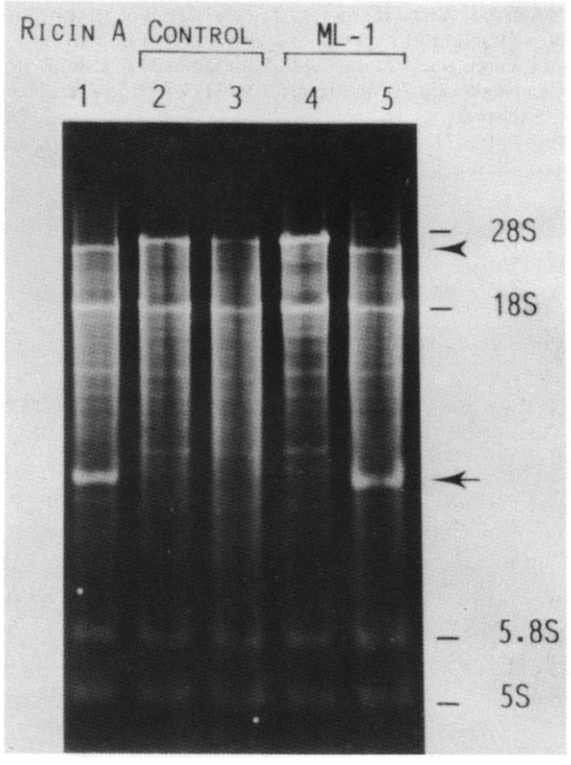

Fig.1. Analysis by gel electrophoresis of rRNA from ML-A-treated ribosomes. Rat liver ribosomes (3.49×10^{-7} M) were incubated without (control) or with ML-A (3.3×10^{-8} M) for 10 min at 37°C. The RNAs were extracted and separated on 2.5% acrylamide-0.5% agarose composite gel before (lanes 2,4) and after the treatment of the RNA with aniline at acidic pH (lanes 3,5). Arrow and arrowhead denote the fragments produced by the ML-A/aniline treatment from 28 S rRNA containing its 3′- and 5′-end, respectively. Lane 1 shows ricin A-chain/aniline treated sample as a marker.

The two newly formed fragments (arrow and arrowhead) appeared to have the same sizes as the corresponding fragments produced by ricin A-chain/aniline (lane 1). The results indicated that ML-A has the same mechanism of action as ricin A-chain on the inactivation of the ribosomes. This

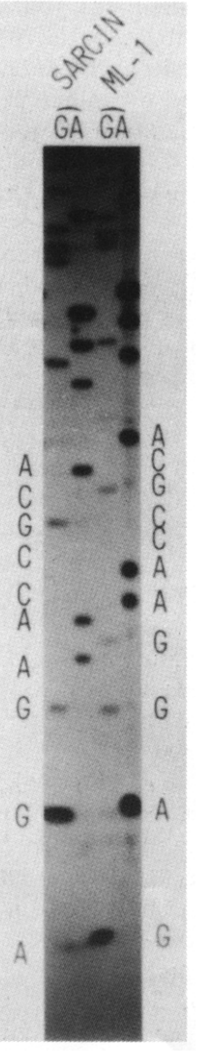

Fig.2. Radioautograph of sequencing gel of the fragment at the 5′-end digested with ribonucleases. The ribosomes were treated with ML-A as in fig.1. The 3′-side fragment (shown with arrow in fig.1) was purified by sucrose density gradient centrifugation. The RNA was treated with bovine alkaline phosphatase and then made radioactive at the 5′-terminal with [γ-^{32}P]ATP and T_4-kinase. The repurified fragment was partially digested with either ribonuclease T1 (G), or ribonuclease U2 (A). The 5′-terminal labeled α-fragment which is produced by α-sarcin was also sequenced as a marker.

point was confirmed by the direct sequencing of the fragment. For this purpose, the smaller fragment (arrow in fig.1) was isolated and its 5′-end was labeled with [γ-^{32}P]ATP and the 5′-terminal nucleotide sequences were determined by the enzymatic method. The 5′-end of the fragment was identified as G-4325 of the 28 S rRNA which is close to the α-sarcin site (fig.2). The results indicate that ML-A cleaves the *N*-glycosidic bond at A-4324 in 28 S rRNA because the β-elimination reaction catalyzed by aniline at acidic pH on the 28 S rRNA isolated from ML-A-treated ribosomes resulted in chain scission at both 3′- and 5′-side of A-4324 giving pG-4325 as the 5′-end of that fragment.

From the above results, we conclude that ML-A inactivates eukaryotic ribosomes by cleaving a single *N*-glycosidic bond at A-4324 of 28 S rRNA in the ribosomes as ricin A-chain does.

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