RNA N-Glycosidase Activity of Ricin A-chain

MECHANISM OF ACTION OF THE TOXIC LECTIN RICIN ON EUKARYOTIC RIBOSOMES*

(Received for publication, February 11, 1987)

Yaeta Endo and Kunio Tsurugi
From the Department of Biochemistry, Yamanashi Medical College, Tamaho, Nakano-ku, Yamanashi 409-38, Japan

The modification reaction of 28S rRNA in eukaryotic ribosomes by ricin A-chain was characterized. To examine whether ricin A-chain release any bases from 28S rRNA, rat liver ribosomes were incubated with a catalytic amount of the toxin, and a fraction containing free bases and nucleosides was prepared from the postribosomal fraction of the reaction mixture by means of ion-exchange column chromatography. Thin-layer chromatographic analysis of this fraction revealed a release of 1 mol of adenine from 1 mol of ribosome. The ribosomes or naked total RNAs were treated with ricin A-chain in the presence of [32P]phosphate, little incorporation of the radioactivity into 28S rRNA was observed, indicating that the release is not mediated by phosphorolysis. Thus, considering together with the previous result (Endo, Y., Mitsui, K., Motizuki, M., and Tsurugi, K. (1987) J. Biol. Chem. 262, 3908-3912), the results in the present experiments demonstrated that ricin A-chain inactivates the ribosomes by cleaving the N-glycosidic bond of A^4324 of 28S rRNA in a hydrolytic fashion.

Ricin is a cytotoxic protein isolated from castor bean that inhibits protein synthesis in intact cells as well as in a cell-free system inactivating large ribosomal subunits (see Ref. 1 for a review). In our previous report (2) it was shown that ricin A-chain, the catalytic subunit of the toxin, enzymatically modifies either or both of the nucleoside residues at positions 4323 and 4324 of 28S rRNA which are close to the α-sarcin cleavage site. The specificity of the toxin for the reaction is very strict: not only are 5S, 6S, 8S, and 18S unaltered but also 1 or 2 nucleoside residues in 28S rRNA is affected. Moreover, the toxin also acts on deproteinized 28S rRNA causing the same modification probably at the same site as in the ribosomes (2).

On the mode of the modification reaction caused by ricin A-chain, we presented a hypothesis (2) that the toxin cleaves the N-glycosidic bond of A^4324 residue in 28S rRNA from the following observations. First, as the result of modification, A^4324 in 28S rRNA becomes resistant to the action of ribonuclease T1, U1, and Phy M. Second, both the 5' and 3' phosphodiester bonds of A^4324 become very unstable to the treatments with dilute alkaline and with aniline at acidic pH.

Now, we present direct evidence that ricin A-chain cleaves the N-glycosidic bond of the residue A^4324 in a hydrolytic fashion.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Quantitation of adenine released from the ribosomes by the action of ricin A-chain

Details are described under “Experimental Procedures.”

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Ribosome* Recoveryb</th>
<th>Amount of base measuredc</th>
<th>Total base released</th>
<th>Molar ratiod</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ade     Gua</td>
<td>Ade       Gua</td>
<td>Ade     Gua</td>
<td>Ade       Gua</td>
</tr>
<tr>
<td>Untreated</td>
<td>2.33    73.6</td>
<td>67.1    ND</td>
<td>ND       ND</td>
<td>ND         ND</td>
</tr>
<tr>
<td>Ricin A-chain treated</td>
<td>1       2.33    72.7</td>
<td>66.3    1.43</td>
<td>ND       1.97</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>2       2.33    71.1</td>
<td>66.5    1.34</td>
<td>ND       1.89</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>3       2.33    75.9</td>
<td>69.2    1.38</td>
<td>ND       1.82</td>
<td>0.78</td>
</tr>
</tbody>
</table>

*The calculations are based on a value for $E_{1%28}^{28}$ of 100 and a molecular weight of 4.3 x 106 (7).

*Calculated from the recovery of [3H]adenine and [14C]guanine.

*Estimated by measuring the relative intensity to the standard spots.

*Released moles of the base per mol of ribosome.

*ND, not detectable.

The 50% ethanol-soluble fraction of the reaction mixture in which rat liver ribosomes were treated with ricin A-chain. The fraction from ricin-treated ribosomes contained a major spot of UV absorbing material which is absent in the fraction from control ribosomes by thin-layer chromatography (Fig. 1A, lanes 2 and 3). The material of the spot was identified to be adenine from its $R_f$ values by two different solvent systems (Fig. 1, A and B). It must be noted that, although there are some minor nucleoside spots on the thin-layer plate (Fig. 1B, lanes 5 and 6), none of them correspond to guanine.

The amount of adenine was quantitated by densitometry using pure adenine as standards and the molar ratio of released adenine to ribosome was calculated (Table I). The result indicates that every ribosome liberated adenine in a nearly stoichiometric ratio (0.78 to 0.84 mol of adenine/1 mol of ribosome). Therefore, considered with the results shown in the previous paper, we conclude that ricin A-chain inactivates ribosomes by cleaving the N-glycosidic bond at A$^{4324}$ but not at G$^{4123}$ in 28 S rRNA.

**Mechanism of Action**—The N-glycosidic bond of the nucleoside residue in 28 S rRNA can be enzymatically cleaved by either phosphorolysis or hydrolysis. Olsnes et al. (6) observed that ricin A-chain inactivated ribosomes simply in Tris/KCl/MgCl₂ medium and hence claimed that ricin A-chain is a hydrolytic enzyme. However, this point has not been formally established as it is possible that the ribosome preparation carries even a trace of phosphate. In the phosphorolytic mechanism, phosphate should be incorporated into nucleoside residue A$^{4324}$ forming ribose 1-phosphate. To test this possibility, ribosomes were treated with ricin A-chain in the presence of $^{32}$P-phosphate and the incorporation of the radioactivity into the 28 S rRNA fraction was measured (Table II). There was little incorporation of phosphate into the 28 S rRNA as it was calculated that less than 1 mol of phosphate/100 mol of modified 28 S rRNA was incorporated. A possibility that the specific radioactivity was diluted by free phosphate associated with ribosomes was ruled out because essentially the same result was obtained when the naked rRNA was incubated, which is expected to carry lesser amounts of phosphate than ribosomes (lower panel). Thus, it is concluded that ricin A-chain does not act as a phosphorolytic enzyme.

So far we have shown that ricin A-chain inactivates eukaryotic ribosomes by cleaving the N-glycosidic bond of A$^{4324}$ in the 28 S rRNA, probably in a hydrolytic fashion. N-glycosidase is a class of enzymes that cleave N-glycosidic bonds in a hydrolytic fashion, and within this group are found enzymes acting on such diverse substrates as uridine (8), NAD⁺ (9),

**FIG. 1. Identification of base liberated from the ribosomes by the action of ricin A-chain.** Bases were isolated from the reaction mixture and separated on silica gel plate with chloroform/methanol/ammonia (A) or 1-butanol/methanol/H₂O/ammonia (B) as solvent. A, lanes 1 and 4: authentic markers; bases from control (lane 2) and ricin-treated ribosomes (lane 3). B, lanes 1–4: standards of adenine and guanine containing various amounts. Lanes 1–4 contain 0.75, 1.13, 1.50, and 1.88 nmol of adenine and 0.66, 0.99, 1.31, and 1.64 nmol of guanine, respectively. Lane 5, bases from control ribosomes; Lane 6, bases from ricin A-chain treated ribosomes; lanes 7–9, authentic markers as shown (Hyp represents hypoxanthine). It should be noted that migration rates of bases and nucleosides in B are a little different from those reported by others (5) who used a cellulose layer plate. Arrows represent the spot released from the ribosomes by the action of ricin A-chain.

**TABLE II**

*Search for incorporation of phosphate into 28 S rRNA during cleavage of the N-glycosidic bond of A$^{4324}$*

Ribosomes or naked total rRNA (34.9 pmol) were incubated with ricin A-chain in the presence of $^{32}$P; in 100 μl of buffer (25 mM Tris-HCl, pH 7.6, 25 mM KCl, and 5 mM MgCl₂). The treatment of ribosomes or naked rRNA with ricin A-chain resulted in 100 and 64% of cleavage of the N-glycosidic bond of A$^{4324}$, respectively. The moles of phosphate incorporated were represented after subtraction of those of the toxin-untreated ribosomes (nonspecific adsorption) which were usually between 2.3 and 3.7 pmol/34.9 pmol of 28 S rRNA.

<table>
<thead>
<tr>
<th>Reaction conditions</th>
<th>Reaction</th>
<th>pmol in 100 μl of reaction mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 ng of ricin A-chain and</td>
<td>Aniline-sensitive rRNA</td>
<td>34.9</td>
</tr>
<tr>
<td>ribosomes, 37°C, 10 min</td>
<td>Phosphate incorporated</td>
<td>0</td>
</tr>
<tr>
<td>10.0 ng of ricin A-chain and</td>
<td>Aniline-sensitive rRNA</td>
<td>34.9</td>
</tr>
<tr>
<td>ribosomes, 37°C, 10 min</td>
<td>Phosphate incorporated</td>
<td>0.2</td>
</tr>
<tr>
<td>10.0 ng of ricin A-chain and</td>
<td>Aniline-sensitive rRNA</td>
<td>22.3</td>
</tr>
<tr>
<td>naked rRNA, 37°C, 60 min</td>
<td>Phosphate incorporated</td>
<td>0.1</td>
</tr>
</tbody>
</table>
and S-adenosylhomocysteine (10). A different group of N-glycosidases which hydrolyze base-sugar bonds in DNA was recently discovered in bacteria (11, 12). They specifically attack DNA containing damaged or nonconventional bases and are believed to function in DNA repair. However, ricin A-chain as demonstrated in this study is totally different from them in the respect that the toxin cleaves only one particular N-glycosidic bond on approximately 7000 present in eukaryotic rRNA.

In a foregoing paper we showed that other toxic lectins, abrin and modeccin, have a similar mode of action on eukaryotic ribosomes as ricin does (2). Recently, we have found that Shiga toxin (13) and the pokeweed antiviral protein (14) also inactivate eukaryotic ribosomes by the same mechanism as ricin. Thus, the RNA N-glycosidase activity found in ricin A-chain seems to be one of the enzymatic activities generally found among the protein toxins that inactivate eukaryotic ribosomes.

REFERENCES