Partial restoration of inactivated ribosomes with sodium borohydride or amino acids

Qing Li, Jun Ling, Wang-yi Liu*

Shanghai Institute of Biochemistry, Academia Sinica, 320 Yue-yang Road, Shanghai 200031, People's Republic of China

Received 18 May 1995; revised version received 1 July 1995

Abstract The aldehyde radical of ribose C_1' at position 4324 in rat liver 28S rRNA generated by RNA *N*-glycosidase was either reduced to an hydroxyl group by sodium borohydride or converted into aldimine through a nucleophilic addition of amino acid used as a primary amine. Analysis of the R-fragment of 28S rRNA by polyacrylamide gel electrophoresis showed that the reduction of aldehyde to an hydroxyl group with sodium borohydride was highly specific. The protein synthesis activity of modified ribosomes was partially restored with the removal of the active aldehyde by sodium borohydride or amino acid. Reduction of aldehyde with sodium borohydride restored 43.1% of the protein synthesis activity. Among the twenty natural amino acids tested, tryptophan and histidine could restore 57.4% and 42.1% of the ribosome activity when brome mosaic virus RNA was used as mRNA. We came to the conclusion that the active aldehyde radical at position 4324 of 28S rRNA in modified ribosome may cause the inactivation of the ribosome for protein synthesis.

Key words: Amino acid; Protein synthesis; Ribosomal RNA; RNA *N*-glycosidase; Sodium borohydride

1. Introduction

Ribosome-inactivating proteins (RIPs) are a group of cytotoxic proteins that catalytically inhibit protein synthesis by affecting the interaction of elongation factors with ribosomes [1–4]. According to the enzymatic mechanisms of action, RIPs can be classified into two types: the first type such as α-sarcin has a novel ribonuclease activity which selectively hydrolyzes the single phosphodiester bond on the 3′ site of the guanosine at position 4325 in rat 28S rRNA [5]. The second type such as the ricin A-chain and trichosanthin is an RNA *N*-glycosidase which specifically cleaves the N–C glycosidic bond of a conserved adenosine at position 4324 among the 4718 nucleotides of rat liver 28S rRNA [6,7]. The acting site of RIPs in two types is located in a highly conserved purine-rich looped 'α-sarcin/ricin domain (S/R domain)' in rat liver 28S rRNA [8], which is involved in the EF-1 dependent binding of aminoacyl-tRNA to the ribosome and EF-2 catalyzing GTP hydrolysis and translocation.

It is known that high concentrations of magnesium ions could restore 20–35% of the activity lost by ricin A-chain treated ribosomes, and that ricin shifted the thermal denaturation curve of 60S subunits towards lower temperatures [9,10]. These facts suggested that inactivation of the 60S subunit by the toxin is associated with, and possibly caused by conformational changes as a result of the cleavage of the N–C glycosidic bond at A4324 in 28S rRNA.

*Corresponding author. Fax: (86) (21) 4338357.

It should be noted that an active aldehyde group was generated at the ribose C_1' of position 4324 in rat 28S rRNA when the ribosome was treated with RNA *N*-glycosidase. Therefore, it is of interest to investigate the effect of the active aldehyde group on protein synthesis activity of the modified ribosome. Since the aldehyde group is very active, it reacts easily with a nucleophilic group to produce a Schiff's base. Thus, the aldehyde group may be one of the factors that cause the conformational changes of 'S/R domain' and the blocking of the binding sites for elongation factors, and thus inactivate the ribosomes. The preliminary results in this communication show that the activity of inactivated ribosome could be partially restored when the aldehyde group was reduced into hydroxyl group with sodium borohydride or blocked with amino acid through a nucleophilic addition.

2. Materials and methods

2.1. Materials

Polyuridylic acid, puromycin, ricin were purchased from Sigma chemical Co., USA. Brome mosaic virus (BMV) was a gift from the Shanghai Institute of Plant Physiology, Academic Sinica. Creatine phosphokinase, phosphocreatine, ATP, GTP and all amino acids were from Shanghai Dong Feng Biochemical Technology Company. [^{3}H]Leucine and [^{3}H]phenylalanine were products from the Shanghai Institute of Atomic Nucleus Research, Academia Sinica. Other chemical reagents were of analytical grade. Ricin A-chain was prepared according to the method of Olsnes [11]. Camphorin and cinnamomin were prepared by the method as described previously [12].

2.2. Preparation of ribosomes

Rat liver 80S ribosomes were prepared according to the method of Spedding [13] and an aliquot containing 200–300 A_{260nm}/ml of ribosome in buffer A (20 mM Tris-HCl, pH 7.6, 100 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 0.25 M sucrose) was stored at −70°C. S100 and pH 5 enzyme were prepared from rat liver as described by Staehelin and Falvey [14] and stored at −70°C in small portions.

2.3. Modification of ribosomes by RNA N-glycosidase

Treatment of ribosomes with RNA *N*-glycosidase was performed according to the method of Endo et al [6]. Three A_{260nm} of ribosomes were incubated with 100 ng RNA *N*-glycosidase (ricin A-chain, camphorin or cinnamomin) at 37°C for 15 min. The modified ribosomes were recovered by ultracentrifugation at 105,000 × *g* for 4 h, and then resuspended in buffer A.

2.4. Reduction of the active aldehyde group with sodium borohydride

The active aldehyde radical at position 4324 in 28S rRNA generated by ricin A-chain action was reduced with sodium borohydride by the method of Randerath et al. [15]. The modified ribosomes were reacted with 10 mmol/l sodium borohydride to reduce the aldehyde group into hydroxyl group, and then assayed the protein synthesis activity in a reconstituted system.

2.5. Blockage of the active aldehyde group with amino acids

The aldehyde radical generated by camphorin or cinnamomin was blocked with amino acids through a nucleophilic reaction according to the method of Cordes and Jencks [16]. The reaction medium contained

SSDI 0014-5793(95)00794-6

Table 1
Partial restoration of camphorin-modified ribosome with amino acids

	Protein synthesis activity (%)				
	Leucine	Tryptophan	Histidine	Aspartic acid	Arginine
Camphorin-modified ribosomes treated with amino acid (A)	41.2	61.3	52.2	31.6	33.0
Camphorin-modified ribosomes (B)	11.0	3.9	10.1	9.8	6.5
Restored activity (C)	30.2	57.4	42.1	21.8	26.4

Three A_{260nm} of rat 80S ribosomes were incubated with 100 ng of camphorin at 37°C for 15 min followed by the treatment with 100 molar excess of amino acid to block the aldehyde at position 4324 in 28S rRNA. The modified ribosomes were recovered by centrifugation at 145,000 × g for 2 h, and then assayed the protein synthesis activity in the reconstituted system of rat liver with brome mosaic virus RNA as mRNA. For each amino acid, it has its own contrast. The protein synthesis activity of the native ribosomes was 100%, (C) = (A) − (B). The data are the avarage value of triplicates.

in a final volume of 30 μl: 20 mM Tris-HCl, pH 7.6, 100 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, and 3 A_{260nm} of camphorin (or cinnamomin)-treated ribosomes. The concentration of amino acids used was about 100-fold higher than that of ribosomes. After incubation at 30°C for 60 min, the reaction mixture was supplemented into the reconstituted system of rat liver to assay the protein synthesis activity of the modified ribosomes.

2.6. Assay for protein synthesis activity in vitro

The protein synthesis activity of the modified ribosomes was assayed in the reconstituted system of rat liver that was prepared by method of Staehalin and Falvey [14]. The reaction mixture contains in 100 μl: 0.1 μmol ATP, 0.04 μmol GTP, 1μmol phosphocreatine, 4 μg creatine phosphokinase, 20 μl of pH 5 enzyme, 20 μl of S100, 5 μCi [^{3}H]leucine or 5 μCi [^{3}H]phenylalanine, 3 A_{260nm} of the modified ribosomes and either 2 μg brome mosaic virus RNA or 10 μg polyuridylic acid as mRNA. After incubation at 37°C for 40 min, the radioactivity incorporated into polypeptide or polyphenylalanine was quantitated by the liquid scintillation counting techniques with a Beckman LS-5801 Liquid Scintillation Counter.

2.7. Extraction, isolation of total rRNAs and aniline reaction

Total rRNAs of the modified ribosomes were extracted from the reaction mixtures with 0.5% sodium dodecyl sulfate and phenol according to the method of Endo and Wool [5]. The recovered rRNAs were treated with acidic aniline (1 M) and analyzed by electrophoresis in either 2.5% acrylamide–0.5% agarose composite gel or 5% polyacrylamide gel.

3. Results

3.1. Restoration of protein synthesis activity of the inactivated ribosome by reduction with sodium borohydride or blockage with amino acids

When the rat liver ribosome was treated with ricin A-chain, its translation activity was severely damaged. Only 3.2% of the protein synthesis activity of the modified ribosome remained as assayed in the reconstituted system with BMV RNA as mRNA. The treatment of ribosome with ricin A-chain led to the depurination at A4324 in 28S rRNA, generating an active aldehyde group at ribose C_1'. In order to study whether the active aldehyde group is involved in the inactivation of the modified ribosome, reduction of the aldehyde group with sodium borohydride was carried out. The experimental results indicated that the protein synthesis activity of the ribosome treated with ricin A-chain was restored up to 43.1–43.9% by treatment with sodium borohydride, while the ribosome not treated with ricin A-chain was almost not influenced by sodium borohydride. This fact showed that the restoration of the activity of the modified ribosome was mainly due to the reduction of aldehyde into hydroxyl group at position 4324 of 28S rRNA with sodium borohydride. In addition, the results also showed that the reactivation of ribosomes treated by ricin A-chain together with sodium borohydride is the same as that of ribosomes treated by ricin A-chain first and then with sodium borohydride.

The results of Table 1 and 2 show that when the aldehyde

Table 2
Partial restoration of intact cinnamomin-modified ribosomes with amino acids

Amino acid	Protein synthesis activity (%)		
	Cinnamomin-modified ribosomes treated with amino acid (A)	Cinnamomin-modified ribosomes (B)	Restored activity (C)
Glycine	35.1	23.8	11.3
Alanine	41.9	23.8	18.1
Leucine	35.5	23.8	11.7
Isoleucine	36.3	23.8	12.5
Methionine	38.4	23.8	14.6
Valine	44.2	23.8	20.4
Phenylalanine$^+$	34.8	23.8	11.0
Proline	32.6	23.8	8.8
Serine	40.9	24.9	16.0
Threonine	44.7	24.9	19.8
Tryptophan	61.5	24.9	36.6
Tyrosine*	34.0	24.9	9.1
Asparagine	32.9	24.9	8.0
Glutamine	45.5	24.9	20.6
Cysteine*	33.0	24.9	8.1
Aspartic acid	41.6	27.3	14.3
Glutamic acid	48.0	27.3	20.7
Arginine	38.6	27.3	11.3
Lysine	50.0	27.3	22.7
Histidine	55.5	27.3	28.2

Three A_{260nm} of rat 80S ribosomes were incubated with 100 ng of intact cinnamomin at 37°C for 15 min, followed by centrifugation at 145,000 × g for 2 h. The recovered ribosomes were resuspended in buffer A, and then treated with 100 molar of different amino acid to block the aldehyde group at position 4324 in 28S rRNA. The modified ribosomes were assayed the protein synthesis activity in the reconstituted rat liver system with polyuridylic acid as mRNA by several amino acids as a group. In each group, it has its own contrast. The restored activity was calculated as (C) = (A) − (B). The protein synthesis activity of the native ribosome is 100%. The data are the average value of the triplicates.

*Tyrosine and cysteine were in 20 molar excess to ribosome.

$^+$The ribosomes blocked with phenylalanine were recovered by centrifugation at 145,000 × g for 2 h, and then assayed for protein synthesis activity in the reconstituted system.

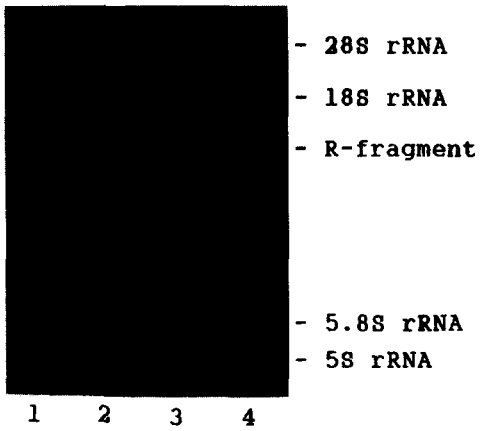

Fig. 1. Specific reaction of sodium borohydride with the aldehyde group of ribose C_1' at position 4324 in rat 28S rRNA generated by the action of ricin A-chain. Two A_{260nm} of rat liver 80S ribosomes were incubated with 100 ng of ricin A-chain followed by treatment with 10 mmol/l (lane 3) or 20 mmol/l (lane 4) of $NaBH_4$, then total rRNAs were extracted and treated by acidic aniline. The resultant rRNAs were analysed by 5% polyacrylamide gel electrophoresis. Lane 1, ribosomal RNAs without treatments; lane 2, ribosomes were treated with ricin A-chain without reduction by sodium borohydride.

group was blocked with amino acid, the activity of the inactivated ribosomes also recovered to some extent. Among the twenty natural amino acids tested, tryptophan and histidine gave higher recovery (36.6–57.4% and 28.2–42.1%) of the protein synthesis activity of the modified ribosome. An explanation may be that tryptophan and histidine have heterocyclic structures similar to a nucleobase. The divergent effects exhibited by various amino acids may indicate that the conformational changes of 'R/S domain' caused by different amino acids are different.

The results of Table 1 were obtained with BMV RNA as mRNA, while that of Table 2 were with polyuridylic acid as mRNA. The difference between the residual protein synthesis activity of the modified ribosome using different RNAs as mRNA was significant. The residual activity with polyuridylic acid as mRNA was higher than that with brome mosaic virus RNA as mRNA. The difference in the restoration of the ribosome activity with different translation templates perhaps was caused by their distinct translational efficiencies.

3.2. *Specificity of the reduction reaction with sodium borohydride*

A ribosome is a complex ribonucleoprotein particle composed of RNAs and proteins. It needs to prove the specificity of reduction of sodium borohydride on the modified ribosome. Ricin A-chain, camphorin and cinnamomin hydrolyze the same N–C glycosidic bond at A4324 in rat 28S rRNA, generating a fragment of about 400 nucleotides from the 3'-end of 28S rRNA called R-fragment after acidic aniline treatment [6,12]. The results of gel electrophoresis showed that the amount of R-fragment decreased after the reduction with sodium borohydride, and no R-fragment emerged when the concentration of sodium borohydride was increased to 20 mmol/l (Fig. 1). It indicated that the reduction of sodium borohydride took place specifically on the C_1' aldehyde group at position 4324 in 28S rRNA.

4. Discussion

When the aldehyde group at position 4324 in 28S rRNA generated by RNA *N*-glycosidase was reduced with sodium borohydride or blocked with amino acid, the protein synthesis activity of the modified ribosome was partially restored. The results was consistent with the known facts that magnesium ions, GTP, and elongation factors could partially restore the translation activity of ricin A-chain or abrin A-chain treated ribosomes [9,17,18]. Perhaps one could explain that a conformational change is induced to improve the interaction of the factor-GTP complexes with the ribosomes. The active aldehyde group may be one of the important factors that cause the conformational change of the 'R/S domain', interfering the binding of elongation factors to the ribosome. Recently, Wool et al. [19] proposed a new translocational model, which demonstrated that GAGA sequence in 'S/R domain' could form a loop through its context G–C pairing. The conserved adenosine at position 4324 at the top of the loop could be recognized and acted on by the ricin A-chain. The change of the 'R/S domain' in vivo induced by the elongation factor is crucial in the conformational transition of the topological structure of the ribosome before and after translocation. When the rat liver ribosome was treated with ricin A-chain, not only an adenine at position 4324 in 28S rRNA was removed, but also the conformation of 'R/S domain' changed. Our results show that the active aldehyde at position 4324 in 28S rRNA generated by RNA *N*-glycosidase exerts significant influence on the biological activity of the ribosome.

It is interesting that camphorin and cinnamomin both exhibited the enzymatic activity to cleave supercoiled DNA into nicked and linear forms. They only act on supercoiled double-stranded DNA but not on the linear form of the same molecule [20].

Acknowledgements: This work is supported by a grant from the National Natural Science Foundation of China and a grant from the Academia Sinica. The authors thank Professor You-Shang Zhang of our institute for his critical reading of the manuscript.

References

[1] Conde, F.P., Fernandez-Puentes, C., Montero, M.T.V. and Vazquez, D. (1978) FEMS Microbiol. Lett. 4, 349–355.
[2] Hobden, A.N. and Cundliffe, E. (1978) Biochem. J. 170, 57–61.
[3] Carrasco, L., Fernandez-Puentes, C. and Vazquez, D. (1975) Eur. J. Biochem. 54, 499–503.
[4] Nolan, R.D., Grasmuk, H. and Drews, J. (1976) Eur. J. Biochem. 64, 69–75.
[5] Endo, Y. and Wool, I.G. (1982) J. Biol. Chem. 257, 9054–9060.
[6] Endo, Y., Mitsui, K., Motizuki, M. and Tsurugi, K. (1987) J. Biol. Chem. 262, 5908–5912.
[7] Zhang, J.S. and Liu, W.Y. (1992) Nucleic Acids Res. 20, 1271–1275.
[8] Chan, Y.L., Endo, Y. and Wool, I.G. (1983) J. Biol. Chem. 258, 12768–12770.
[9] Cawley, D.B., Hedblom, M.L. and Houston, L.L. (1979) Biochemistry 18, 2648–2654.
[10] Paleologue, A., Reboud, J.P. and Reboud. A.M. (1986) FEBS Lett. 208, 373–378.
[11] Olsnes, S. (1978) Methods Enzymol. 50, 330–335.
[12] Ling, J., Liu, W.Y. and Wang, T.P. (1995) Biochim. Biophys. Acta., in press.
[13] Spedding, G. (1990) in: Ribosomes and Protein Synthesis: A Practical Approach (Spedding, G., Ed.) pp. 1–29, IRL, Oxford, New York.

[14] Staehelin, T. and Falvey, A.K. (1971) Methods Enzymol. 20, 433–446.
[15] Randerath, E., Yu, C.T. and Randerath, K. (1972) Anal. Biochem. 48, 172–198.
[16] Cordes, E.H. and Jencks, W.P. (1963) J. Am. Chem. Soc. 85, 2843–2848.
[17] Olsnes, S., Fernandez-Puentes, C., Carrasco, L. and Vazquez, D. (1975) Eur. J. Biochem. 60, 281–288.
[18] Skorve, J., Abraham, A.K., Olsnes, S. and Pihl, A. (1977) Eur. J. Biochem. 79, 559–564.
[19] Wool, I.G., Glück, A. and Endo, Y. (1992) Trends Biochem. Sci. 17, 266–269.
[20] Ling, J., Liu, W.Y. and Wang, T.P. (1994) FEBS Lett. 345, 143–146.