INVOLVEMENT OF SULFHYDRYL GROUPS IN THE ACTIVATION OF LATENT RIBONUCLEASE OF RAT LIVER POLYRIBOSOMES

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1. Introduction

The stability of isolated rat liver polyribosomes decreases greatly after treatment with certain sulfhydryl blocking agents [1] or after Sephadex gel filtration [2]. In the present paper evidence is presented that in both cases the decreased stability of the polysomes is due to activation of latent ribonuclease involving the blocking or oxidation of sulfhydryl groups. The data suggest that the ribonuclease associated with rat liver polyribosomes, like the alkaline ribonuclease of the cell sap [3, 4], is present in a complex with an inhibitor which requires free sulfhydryl groups for its activity.

2. Materials and methods

Polysomes were prepared as described by Earl and Morgan [5] from livers of male Wistar rats (150–200 g), and stored as pellets at −70°. The pellets were suspended in ice-cold TKM buffer (0.05 M tris-HCl buffer, pH 7.6, 0.025 M KCl, 0.005 M $MgCl_2$), and gently dispersed in a Potter-Elvehjem teflon-glass homogenizer.

Sucrose density gradient analysis was carried out by layering aliquots of the polysome preparations onto linear 10–30% gradients made up in TKM buffer. After centrifugation for 40 min at 39,000 rpm in the SW 39 rotor of the Spinco model L2 ultracentrifuge the sedimentation pattern was determined by measuring the optical density at 254 nm in a flow cell (Isco model UA-2 analyzer). RNAase activity was assayed by measuring the increase in optical density at 260 nm in the cold trichloroacetic acid soluble fraction, as described by Utsunomiya and Roth [6]. Protein was determined by the method of Lowry et al. [7] with bovine serum albumin as reference standard.

3. Results and discussion

The data in fig. 1 demonstrate that filtration of a polysome preparation through Sephadex G-50 resulted in a rapid breakdown to monosomes and disomes as previously found [2], while the unfiltered control showed a typical polysome sedimentation pattern. Moreover, it is seen that addition of reduced glutathione (GSH) immediately after gel filtration abolished the decrease in the stability of the polysomes which otherwise occurred. The data in table 1 demonstrate that the disaggregation of the polysomes

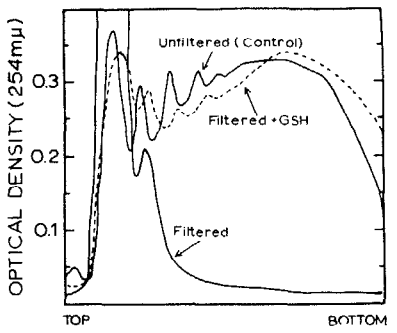

Fig. 1. Effect of reduced glutathione (GSH) on the stability of polysomes filtered through Sephadex G-50. Polysomes dispersed in TKM buffer were filtered through Sephadex G-50 as described in legend to table 1. After incubation at 37° for 10 min the preparation was submitted to sucrose density gradient analysis. To one of the samples 1 mM GSH was added immediately after filtration.

Table 1
Effect of various compounds on the stability and RNAase activity of Sephadex filtered polysomes.

Conditions	Polysome sedimentation pattern	RNAase activity (% of control)
Unfiltered (control)	Normal	100
Filtered	Disaggregation	1410
Filtered + glutathione (1 mM)	No disaggregation	87
Filtered + dithiothreitol (1 mM)	No disaggregation	180
Filtered + EDTA (0.1 mM)	No disaggregation	153
Filtered + KCN (2 mM)	No disaggregation	133
Filtered + cysteine (1 mM)	Disaggregation	1580
Filtered + 2-mercaptoethanol (1 mM)	Disaggregation	1370

Polysomes dispersed in TKM buffer were passed through a Sephadex G-50 column (1.5 × 30 cm) equilibrated with TKM buffer. The polysomes were eluted with TKM buffer, and the compounds indicated were added. After incubation at 37° for 10 min the sedimentation pattern was analyzed. The enzyme activity was measured according to Utsunomiya and Roth [6], except that the incubation time was 5 hr.

following filtration on Sephadex is associated with a 14-fold increase in the activity of the RNAase present in the polysome preparation, and that this increase is abolished by glutathione. Dithiothreitol, EDTA and KCN likewise abolished the filtration-induced instability of the polysomes and largely prevented the associated increase in ribosomal RNAase activity. Significantly, cysteine and 2-mercaptoethanol which failed to prevent the disaggregation of the polysomes also failed to prevent the activation of the RNAase (table 1).

The striking correlation between the disaggregation of the polysomes and the activity level of the ribosomal RNAase strongly supports our previous suggestion [2] that the decreased stability of rat liver polyribosomes after Sephadex G-50 filtration is due to activation of latent ribosomal RNAase. The fact that the enzyme activation could be prevented by GSH, dithiothreitol, EDTA and KCN, compounds known to prevent or reverse the oxidation of sulfhydryl groups, indicates that the filtration-induced activation of the RNAase involves oxidation of sulfhydryl groups. The reason why cysteine and 2-mercaptoethanol had no effect is not apparent.

The ability of GSH, dithiothreitol, EDTA and KCN to prevent the filtration-induced activity of RNAase cannot be due to a direct inhibiting effect on the enzyme as such. This follows from the data in table 2, showing that none of these compounds inhibited appreciably the activity of RNAase which had been solubilized from the polysomes.

It has previously been shown by several authors that the cell sap of various tissues from higher animals contains an inhibitor of alkaline RNAase [3, 4, 8–10]. The inhibitor which forms a complex with the RNAase, is a protein requiring a free SH group for its activity [4]. Thus, sulfhydryl reagents block the action of the inhibitor and induce activation of the RNAase [3, 4]. The results in table 3 suggest that a similar situation obtains in the isolated polysomes. It is apparent that the SH-blocking agents *p*-chloromercuribenzoate (PCMB), *N*-ethylmaleimide (NEM) and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) caused disaggregation of the polysomes, in agreement with previous reports [1]. Concurrently, a strong activation of the RNAase

Table 2
Effect of thiols, EDTA and KCN on solubilized polysomal RNAase.

Compounds added	Enzyme activity (% of control)
No addition (control)	100
Glutathione (1 mM)	73
Dithiothreitol (1 mM)	87
Cysteine (1 mM)	83
2-mercaptoethanol (1 mM)	65
KCN (2 mM)	90
EDTA (0.1 mM)	87

As source of soluble RNAase the 105,000 *g* supernatant from a Sephadex G-50 filtered polysome preparation was used. The enzyme activity was measured after incubation for 5 hr.

Table 3
Ability of sulfhydryl blocking reagents to activate the RNAase of polysomes.

Compounds added	Polysome sedimentation pattern	RNAase activity (% of control)
No addition (control)	Normal	100
p-chloromercuribenzoate (1 mM)	Disaggregation	1500
N-ethylmaleimide (1 mM)	Disaggregation	1340
5,5'-dithio-bis(2-nitrobenzoic acid) (1 mM)	Disaggregation	1330
Cystamine (1 mM)	Disaggregation	710

Polysome preparations were mixed with the compounds indicated. After incubation for 10 min at 37° the sedimentation pattern was analyzed. The RNAase activity was measured after incubation for 5 hr.

present in the polysome preparation occurred. The fact that the weakly reactive agent cystamine had similar effects indicates that the sulfhydryl groups involved are highly reactive.

The present data are consistent with the view that the RNAase associated with rat liver polysomes is present in a complex with an inhibitor, requiring SH groups for its activity. It seems unlikely that the sulfhydryl groups involved in the activation of ribosomal RNAase belong to ribosomal proteins or to the proteins known to be associated with mRNA [11, 12]. Thus, it was found that RNAase solubilized from polysomes was activated to the same extent by PCMB and Sephadex filtration, as was the RNAase of the whole polysome preparation (data not shown). The results suggest that actually an RNAase-inhibitor complex had been liberated from the polysomes.

Whether the RNAase of rat liver polysomes represents a contamination from the cytoplasm is not known. The fact that it has not been possible, in spite of considerable efforts, to isolate polysomes free of RNAase, raises the question whether the enzyme is normally associated with the polysomes and plays a role in cellular control of protein synthesis. If this is the case small molecular sulfhydryl compounds, e.g. reduced glutathione, might be involved in the regulatory mechanism.

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