DECREASED STABILITY OF RAT LIVER POLYRIBOSOMES AFTER GEL FILTRATION ON SEPHADEX

P.EKER, R.WEIDENMÜLLER* and A.PIHL
Norsk Hydro's Institute for Cancer Research, Norwegian Radium Hospital, Montebello, Oslo, Norway

Received 26 May 1970

1. Introduction

In recent experiments we have observed that when preparations of rat liver polysomes are filtered through a Sephadex G-50 gel, the stability of the polysomes, as judged by sucrose density-gradient analysis, is markedly decreased. In the present communication data are presented indicating that this decrease in stability after gel filtration is due to activation of latent RNAase associated with the polysomes. It is suggested that the activation is due to the removal of an RNAase inhibitor of low molecular weight.

2. Materials and methods

2.1. *Polysome preparation*

Polysomes were prepared as described by Earl and Morgan [1] 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.

2.2. *Sucrose density-gradient analysis*

Aliquots of the polysome preparations were layered onto linear 10–30% gradients made up in TKM buffer. After centrifugation for 45 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 mμ in a flow cell (ISCO Model UA-2 analyzer).

2.3. *In vitro protein synthesis*

The incorporation of L-phenylalanine-^{14}C was measured in the amino acid incorporating system described by Perani et al. [2] with minor modification. The concentration of tris-HCl buffer was 35 mM, the magnesium chloride concentration 6.5 mM, and ammonium chloride was added to a final concentration of 50 mM. When present, the concentration of poly U was 50 μg per reaction mixture (250 μl). After incubation for 10 min at 37° the reaction was stopped by adding 0.25 ml of ice-cold 10% trichloroacetic acid, containing unlabelled phenylalanine (20 mM). After centrifugation the sediment was suspended in 5% trichloroacetic acid, kept at 90° for 15 min, and rapidly cooled to 0°. The precipitated material was collected on Millipore filters and extensively washed with ice-cold 5% trichloroacetic acid and ethanol.

2.4. *Measurement of radioactivity*

Radioactivity was measured with a liquid scintillation counter. The scintillation fluid consisted of toluene, containing 0.4% 2,5-diphenyloxazole (PPO) and 0.005% 1,4-di(2-(5-phenyloxazolyl)) benzene (POPOP).

2.5. *Analytical methods*

RNAase activity was measured according to Utsunomiya and Roth [3]. Protein was determined by the method of Lowry et al. [4], with bovine serum albumin as reference standard.

* Fellow of the Norwegian Cancer Society (Landsforeningen mot Kreft).

3. Results and discussion

In the present work the polysomes were prepared according to the method of Earl and Morgan [1] which involves the use of ammonium chloride. This procedure is reported to increase the stability of the polysomes by removing most of the RNAase usually associated with polysome preparations. In agreement with this it was found (upper part of fig. 1) that when the polysome preparations were incubated for 30 min at 37°, only a moderate fragmentation occurred. This effect is most likely due to residual RNAase activity. However, similar incubation of a polysome preparation filtered through Sephadex G-50 gel resulted in an almost complete fragmentation to monomeric and dimeric ribosomes (bottom part of fig. 1).

The polysome preparations studied in fig. 1 were subsequently tested in an *in vitro* protein-synthesizing system. The data in table 1 demonstrate that polysomes which had been filtered through Sephadex G-50 and then incubated at 37° for 30 min exhibited a strongly reduced ability to support protein synthesis. This was to be expected from the sedimentation profile of the preparation (fig. 1). It is apparent that addition of poly U to the system fully restored its ability to synthesize protein. This finding shows that the ribosomes as such were intact and that all factors necessary for their attachment to the synthetic messenger were present in the system. These results, together with the sedimentation studies, demonstrate that the gel filtration resulted in a striking decrease in the stability of the polysomes. Furthermore, they indicate that the instability is due to an increased cleavage of the mRNA chain.

Direct evidence that the fragmentation of the gel filtered polysomes involves a cleavage of the messenger is found in the experiments presented in fig. 2.

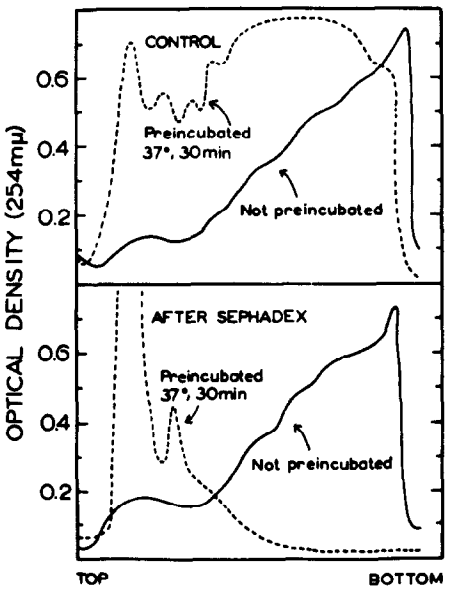

Fig. 1. Density-gradient analyses of polysomes before (control) and after gel filtration on Sephadex G-50. Polysomes dispersed in TKM buffer were passed through a Sephadex G-50 column (1.5 X 30 cm) equilibrated with TKM buffer. The polysomes, eluted with TKM buffer, were collected and the sedimentation pattern was analyzed as described in the text, using a 10–30% linear sucrose gradient.

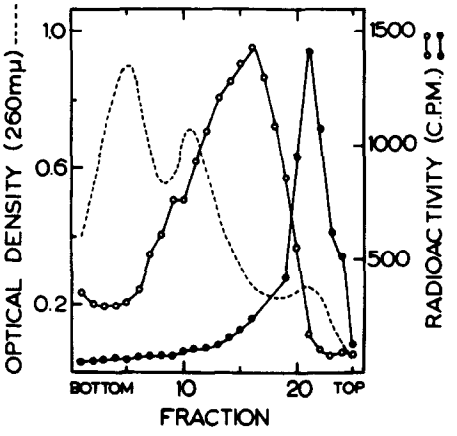

Fig. 2. Sucrose density-gradient analyses of labelled RNA from ribosomes. Polysomes were isolated from the livers of rats injected with ^{3}H-orotic acid (1 mCi) 30 min prior to sacrifice. The preparation was passed through a Sephadex G-50 column, and the RNA deproteinized [6] before and after incubation of the polysomes at 37° for 30 min. The samples were layered on top of a 10–30% sucrose gradient made up in 0.01 M triethanolamine, pH 7.5 containing 0.02 M EDTA, 0.15 M KCl and 2.0 M urea, and centrifuged for 12 hr at 40 000 rpm in the SW 40 rotor. After centrifugation, fractions were collected from the bottom of the tubes for analyses of radioactivity and optical density. Radioactivity of RNA immediately after filtration (o—o), and after incubation for 30 min at 37° (•—•). The dashed line denotes the optical density of the total RNA.

Table 1
Effect of Sephadex filtration on the ability of polysomes to support protein synthesis.

Conditions	Incorporation of ^{14}C-phenylalanine into protein* (cpm/μg protein)			
	Before Sephadex filtration		After Sephadex filtration	
	– Poly U	+ Poly U	– Poly U	+ Poly U
No preincubation	90	169	107	197
Preincubation at 37° for 30 min	88	161	35	202

* Conditions as described in the text.

Here the RNA was isolated before and after incubation at 37° for 30 min of gel filtered polysomes, and the sedimentation pattern of the rapidly labelled RNA fraction was analyzed. The finding that incubation of the filtered polysomes resulted in marked displacement of the labelled RNA from dense to more light regions of the gradient, demonstrates that a cleavage of the mRNA into shorter fragments had indeed occurred.

The most probable explanation of the present finding is that the decreased stability of polysomes after gel filtration is due to activation of latent RNAase. Attempts to demonstrate this by direct measurements of RNAase activity were unsuccessful. Thus, with yeast RNA as substrate, no measurable RNAase activity was detected, either before or after Sephadex filtration. This finding, however, does not preclude the presence of RNAase in the polysome preparations. It may well be that RNAase is associated with the polysomes in such a way that added substrate is not readily accessible to the enzyme.

Activation of RNAase of the polysome preparations by Sephadex filtration could be brought about by the removal of a substance inhibiting the enzyme. If this is the case, the inhibitor must be of low molecular weight since decreased stability of polysomes was observed also after filtration through Sephadex G-25, G-15, and G-10. Since Sephadex G-10 is reported to exclude substances of molecular weight greater than about 700, the inhibitor assumed to operate in the present experiments cannot be identical with the previously described cellular RNAase inhibitors which are known to be substances of high molecular weight [5]. Attempts to isolate and characterize the probable inhibitor substance are now in progress.

References

[1] D.C.N.Earl and H.E.Morgan, Arch. Biochem. Biophys. 128 (1968) 460.
[2] A.Perani, B.Parisi, L.de Carli and O.Ciferri, Biochim. Biophys. Acta 161 (1968) 223.
[3] T.Utsunomiya and J.S.Roth, J. Cell. Biol. 29 (1966) 395.
[4] O.H.Lowry, N.J.Rosebrough, A.L.Farr and R.J.Randall, J.Biol. Chem. 193 (1951) 265.
[5] A.A.M.Gribnau, J.G.G.Schoenmakers and H.Bloemendal, Arch. Biochem. Biophys. 130 (1969) 48.
[6] S.Olsnes, manuscript in preparation.