AN IMPURITY IN RNA PREPARATIONS THAT INTERFERES WITH ASSAYS FOR RIBONUCLEASE AND RIBONUCLEASE INHIBITOR

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While studying the effects of different RNA preparations on the activity of ribonuclease (RNase) inhibitor (6,7) in rat liver supernatant fraction, it was noted that under identical conditions, use of a sample of RNA prepared in our laboratory according to the method of Crestfield, Smith and Allen (1), (CSA-RNA), resulted in a much higher inhibition of crystalline pancreatic RNase than when a commercial (Schwarz) RNA sample was the substrate. While this work was being carried out, a communication from Eichel (4) indicated that the use of another commercial preparation (Pabst) resulted in the complete inactivation of RNase inhibitor and furthermore, was partially inhibitory to RNase itself. In view of the obvious importance of these observations to the reliable assay of both RNase and RNase inhibitor activity, the effect of various substrates has been examined in more detail.

RNA was obtained from Schwarz BioResearch Inc. and Pabst Laboratories. An analysis of the preparation of CSA-RNA used has been published (5). The method of assay for RNase and RNase inhibitor have been described (3,7). The reagent used to precipitate unhydrolyzed RNA was different, however, consisting of 1 N HCl in 76 % ethanol and containing 0.5 % lanthanum chloride. Rat liver supernatant fraction was prepared by homogenization of rat liver in 9 volumes of ice-cold 0.25 M sucrose solution, followed by centrifugation at 60,000 x g for one hour. The supernatant fraction was used without further purification as a source of RNase inhibitor.

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RNase Inhibitor Activity with Different Substrates and the Effect of EDTA:

The results of two typical experiments are shown in Table I. The following points should be noted :

1) The activity of RNase was about the same with either Schwarz RNA or CSA-RNA[1] ; however, with Pabst RNA as substrate the same quantity of enzyme exhibited only slightly more than half this activity (Table I, part A).

2) Addition of EDTA did not affect the RNase activity with either Schwarz or CSA-RNA as substrates but it did increase the activity with Pabst so that it now became the same as with the other two substrates (Table I, part B).

3) In experiment 1 the inhibition of 0.05 ugm of crystalline pancreatic RNase by 0.05 ml of RNase inhibitor was 75 % when CSA-RNA was used and only 9 % with Schwarz RNA as substrate. With Pabst RNA no inhibition was observed and, in fact, there was an increase in RNase activity of 10 % compared to the control. This increase, which is consistently observed under these conditions, is due to release of latent RNase activity present in the supernatant fraction,(8) by the impurity in the Pabst RNA.

4) Upon addition of EDTA, the inhibition of pancreatic RNase by RNase inhibitor is now the same with Schwarz RNA and CSA-RNA and only slightly less with Pabst RNA (Table I part B, exp. 1) . Additional experiments (Table I, part B, exp. 2) showed that by altering the ratio of enzyme to inhibitor and increasing somewhat the amount of EDTA, equal, and probably maximal inhibitions could be obtained with all three substrates.

In view of the effect of EDTA, it appears likely that the impurity in RNA is a metal ion, probably copper in the Pabst RNA, but possibly some other ion in Schwarz RNA. The firm binding of metallic ions by RNA has been well documented (10). Copper appears logical, for in addition to inactivating RNase inhibitor, it is one of the few relatively common ions that is strongly inhib-

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[1] This is not true for all substrate concentrations or quantities of crystalline pancreatic RNase; for example, as the amount of enzyme is decreased from 0.1 ugm (with 1 % RNA) the activity with CSA-RNA becomes progressively less than with Schwarz RNA.

Table I
The Activity of Rat Liver Ribonuclease Inhibitor with Different RNA Substrates with and without added EDTA

Substrate and additions	Activity (O.D. corrected for blank)	Inhibition %	Activity (O.D.corrected for blank)	Inhib. %
A	Exp. 1*		Exp.2**	
1 % CSA-RNA + RNase	.205		.425	
" " " + RNase inhibitor	.046	77.5	.155	63.5
1 % Schwarz RNA + RNase	.234		.422	
" " " + RNase inhibitor	.213	9.0	.382	9.5
1 % Pabst RNA + RNase	.132		.279	
" " " + RNase inhibitor	.146	-10.6	.307	-10.0
B Plus EDTA				
1 % CSA-RNA + RNase	.194		.435	
" " " + RNase inhibitor	.017	91.2	.108	75.2
1 % Schwarz RNA + RNase	.241		.442	
" " " + RNase inhibitor	.022	90.8	.157	64.5
1 % Pabst RNA + RNase	.210		.392	
" " " + RNase inhibitor	.091	56.6	.129	67.1

* In exp. 1, 1 ml of 1 % RNA, 1 ml of Veronal-acetate buffer pH 7.8, 0.05ugm. RNase, 0.05 ml supernatant fraction in a total volume of 3.0 ml incubated for 30 min. at 37° . In part B,0.3 ml of 0.1 % EDTA present.

** In exp. 2, 0.1 ugm RNase and 0.09 ml of supernatant fraction used; other conditions the same. In part B , 0.12 ml of 0.05 M EDTA present.

itory to pancreatic RNase at low concentrations (11). On the other hand, it is only moderately inhibitory to RNase in the crude supernatant fraction of rat liver(9). The impurity is not removed from RNA by exhaustive dialysis but can be removed by dialysis against EDTA solutions. It does not exert its inhibitory effect unless (or until) the RNA is considerably degraded by RNase, this degradation presumably releasing the metallic ion. Further data on this phenomenon as well as assays for the metal ion will be reported in detail elsewhere.

The presence of variable amounts of an impurity in some RNA preparations, interfering with the assay for both RNase and RNase inhibitor, can explain some discrepancies in the literature with respect to alkaline RNase activity.

To mention only one example, deLamirande and co-workers report the ratio of acid RNase to alkaline RNase in rat liver to be about 1 to 1, while in our laboratory this ratio is always approximately 5 to 1. It is obvious that, depending on the amount and character of the impurity present in RNA preparations, wide variations in RNase activity, or in RNase inhibitor activity may be obtained. Since in most cases EDTA does not appear to directly affect RNase activity, the use of optimum amounts in assays for RNase and RNase inhibitor would appear advisable.

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