Solubilization in formamide protects RNA from degradation

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Degradation by RNase is a major concern when handling RNA samples. The omnipresent enzyme (fingertips, dust etc.) is heat-stable and does not require cofactors for its enzymatic activity. This makes it difficult to inactivate RNase in RNA samples. Although there are several isolation methods yielding RNase-free RNA (1, 2), precautions have to be undertaken to avoid RNase contamination at the final step of isolation, i.e. solubilization of RNA. The common practice is to solubilize RNA in diethyl pyrocarbonate (DEPC)-treated water (1, 2). The solubilized RNA is susceptible, however, to accidental contamination with RNase during handling and storage of samples. In addition, long-term storage of water-solubilized RNA samples requires temperature as low as −70°C to prevent degradation of RNA (1, 2).

In an attempt to improve handling of RNA samples, formamide was tested as a solubilizing agent for RNA. Samples solubilized in formamide were stored at −20°C for more than one year. During this period of time, they were repeatedly thawed and subjected to Northern blotting or polymerase chain reaction and no degradation of RNA was detected. The effectiveness of formamide in suppressing RNase activity and its use in long-term storage of RNA is shown in Figure 1. The depicted RNA was isolated from MCF-7 cells, solubilized either in formamide or in DEPC-treated water and incubated with RNase (5−250 μg/ml) for 30 min at room temperature. Following incubation, the samples were applied directly to agarose-formaldehyde gel and electrophoresed. The incubation in a formaldehyde reaction mix before application of RNA onto the agarose gel was not performed. The preliminary experiments indicated that in this water containing reaction mix RNase degrades RNA before being itself inactivated by formaldehyde. Electrophoretically separated RNA was blotted to a Nytran hybridization membrane by a one-hour downward alkaline capillary transfer (3). The RNA immobilized on the membrane was stained with methylene blue and photographed (Figure 1, top). In samples solubilized in formamide, undegraded ribosomal bands were observed at RNase concentrations up to 50 μg/ml. At the RNase concentration of 250 μg/ml, a substantial degradation of both 18S and 28S ribosomal bands was observed. RNA solubilized in water was completely degraded, and did not stain with methylene blue, even at the lowest (5 μg/ml) concentration of RNase.

Following methylene blue staining, the membrane was hybridized with β-actin cDNA. A 2 kb band (Figure 1, bottom) corresponding to β-actin mRNA was observed in samples solubilized in formamide and incubated with up to 50 μg/ml of RNase. The gradual decrease in band intensity, however, may suggests a partial degradation of RNA at RNase concentrations >10 μg/ml. Near total degradation of RNA was observed in a sample incubated with 250 μg/ml of RNase. It is not clear, however, if the RNA degradation occurred during incubation in formamide or during the early stage of electrophoretic separation when RNA samples, together with RNase, were entering the aqueous environment of the agarose gel. No hybridization signal was observed in samples solubilized in water and incubated with RNase, indicating a complete digestion of β-actin mRNA. Control samples in this experiment were stored in formamide at −20°C for eleven months (lanes 1 and 2) and one month (lanes 11 and 12) or in water at −70°C for one month (lanes 9 and 10) . No degradation of ribosomal RNA or β-actin mRNA was observed in samples stored in formamide at −20°C either for eleven months or one month. Protection of RNA from degradation by RNase was observed using formamide obtained from several

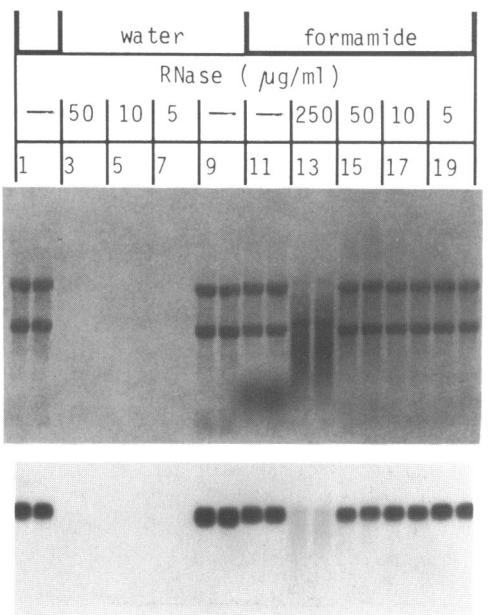

Figure 1. Northern blot analysis of total RNA solubilized in water or formamide and incubated with RNase. Total RNA was isolated from MCF-7 cells by the single-step method (4) using TRI Reagent (Molecular Research Center, Inc., Cincinnati, OH). The RNA was solubilized in DEPC-treated water and stored for one month at −70°C (lanes 3−10) or solubilized in formamide and stored at −20°C for eleven months (lanes 1 and 2) or for one months (lanes 11−20). Aliquotes of total RNA (4.3 μg) were incubated for 30 min at room temperature in 16 μl of water or in 16 μl of formamide with 3 μg (250 μg/ml), 0.6 μg (50 μg/ml), 0.12 μg (10 μg/ml) and 0.06 μg (5 μg/ml) of RNase (bovine pancreas RNase A, Sigma, St Louis, MO) as indicated in the Figure 1. Following incubation, RNA was electrophoresed in agarose (1%)-formaldehyde gel, transferred to a Nytran (Schleicher & Schuell, Keene, NH) membrane, stained with methylene blue (top) and hybridized with nick-translated β-actin cDNA (bottom) as described (3). Dark spots in the low-molecular weight region of lanes 11 and 12 correspond to bromophenol blue.

major manufacturers. However, for a long-term storage a stabilized formamide (Molecular Research Center, Inc., Cincinnati, OH) was used. During experiments, up to 4 mg of RNA was solubilized in 1 ml of formamide. No attempt was made to estimate saturating concentration of RNA in formamide. RNA can be readily precipitated from formamide by the addition of four volumes of ethanol (results not shown).

An additional advantage of formamide as a solubilizing agent for RNA is that high sample volume (up to 50% of the volume of the formaldehyde reaction mix) can be applied to the formaldehyde-agarose gel. In contrast, using water-solubilized samples the sample volume cannot exceed 20% of the volume of the reaction mix (1, 2). The remaining 80% of the volume of the reaction mix consists of formamide (50%), formaldehyde (16%), glycerol (5–10%) and buffer concentrate (5%).

In conclusion, formamide has several advantages over water as a solubilizing agent for RNA. It effectively protects RNA from degradation by RNase, allows for a long-term storage at −20°C and increases the sample volume which can be applied onto a formaldehyde-agarose gel.

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