Comparison of the Influence of Spermidine and Monovalent Salts on RNA Synthesis

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The influence of spermidine and salts on the RNA synthesis was compared.
1. Spermidine as well as salts stimulate the RNA synthesis, catalyzed by the RNA nucleotidyl-transferase and directed by native, double-stranded calf thymus DNA.
2. However, for maximal synthesis both are necessary, because salts prevent the precipitation of the DNA at optimal spermidine concentration (10 mM).
3. Spermidine stimulates the RNA synthesis by creating new RNA chains. On the contrary salts stimulate the RNA synthesis by elongation of the RNA chains.
4. The inhibition of the RNA synthesis by rifampicine, added 5 min after the start of the RNA synthesis, is strongly intensified in the presence of spermidine or high salt concentrations.

It is well known that RNA synthesis, catalyzed by RNA nucleotidyl-transferase and directed by native, double-stranded DNA, is stimulated in the presence of monovalent salts or spermidine. The RNA synthesis continues for a long time in the presence of high salt concentration or spermidine and does not show the plateau kinetics, which is typical of low salt concentration. Besides this, the RNA synthesis is reactivated after reaching the plateau kinetics by the addition of spermidine or salts.

In the following experiments we have compared the effect of salts and spermidine on the RNA synthesis. In addition we have studied the inhibition of the RNA synthesis by rifampicine under these conditions.

For these investigations we worked with singly and doubly labeled ATP.

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In the cases where doubly labeled ATP was used we had to change the washing procedure. The incubations were stopped after the addition of 0.2 ml of a bovine serum albumin solution (0.3%) by 5 ml ice-cold trichloroacetic acid (5%). The samples were kept in ice for 10 min and centrifuged thereafter. The pellets were dissolved in 0.3 ml NaOH (0.2 M) and again precipitated with 5 ml trichloroacetic acid. This washing procedure was repeated twice. The pellets were then collected on membrane filters as described above.

Rifampicine, used in our experiments in a concentration which caused an inhibition of 40–60%, was given with enzyme before the start of the RNA synthesis by the addition of DNA.

The triphosphates ATP, GTP, UTP and CTP were purchased from Boehringer, Mannheim; (8-14C) ATP and ADP from Schwarz BioResearch, Orangeburg, N.Y.; (β-32P)ATP from the Radiochemical Centre, Amersham, England; spermidine-3-HCl from Calbiochem, Los Angeles. Rifampicine was a gift from Ciba AG, Wehr/Baden.

**Results**

Fig. 1 shows the influence of increasing amounts of KCl and (NH₄)₂SO₄ on the RNA synthesis, both, in the absence and in the presence of spermidine.

Spermidine (10 mM) added to a reaction mixture with low salt concentration inhibits the RNA synthesis. By increasing the amount of salt the RNA synthesis is inhibited no longer, but stimulated instead. Maximal synthesis takes place at about 160 mM KCl or 40 mM (NH₄)₂SO₄; higher salt concentrations lead to an inhibition again.

In order to determine the optimal relation between spermidine and salt concentration we carried out an experiment, the result of which is represented in fig. 2. It can be seen that a spermidine concentration of about 5–10 mM and a KCl concentration of 160 mM show an optimal effect on the RNA synthesis.

We repeated a similar experiment using 40 mM (NH₄)₂SO₄ and native, double-stranded DNA as well as heat-denatured, single-stranded DNA as primers. Fig. 3 demonstrates the result. The RNA synthesis is optimally stimulated in the presence of 40 mM (NH₄)₂SO₄ and 10 mM spermidine. This experiment shows furthermore that the inhibition at optimal spermidine concentration and low salt concentration or in the presence of salt and high spermidine concentration is independent of the primer. However, the stimulation of the RNA synthesis was only found, as it is well known, using native, double-stranded DNA as primer.
Fig. 3. Influence of increasing amounts of spermidine on the RNA synthesis in the absence and presence of (NH₄)₂SO₄ using native, double-stranded and heat-denatured DNA. Incubation mixture see fig. 1; (NH₄)₂SO₄ (40 mM) and spermidine (10 mM) as indicated. □—□ native DNA; ■—■ native DNA and (NH₄)₂SO₄; ▼—▼ heat-denatured DNA; ▼—▼ heat-denatured DNA and (NH₄)₂SO₄.

Kinetics

In these experiments we worked with doubly labeled ATP; the RNA synthesis was measured by the incorporation of (8-¹⁴C)AMP; we determined the number of RNA chains by the incorporation of (γ-³²P)ATP.

Fig. 4 shows the kinetics of the RNA synthesis at low salt concentration (control), at high salt concentration (320 mM KCl) and in the presence of spermidine (10 mM).

The control shows the typical plateau kinetics of the incorporation of AMP at low salt concentration. In the presence of spermidine or high salt concentration the RNA synthesis (AMP incorporation) continues without plateau. In the case of high salt concentration a lag phase becomes visible.

A remarkable difference was found, however, in the incorporation of ATP using spermidine or high salt concentration in the incubation mixture. In the presence of spermidine the incorporation of ATP is stimulated as well as the incorporation of AMP. However, in the presence of this high salt concentration only the incorporation of AMP is stimulated, whereas the incorporation of ATP is rather diminished; besides this the velocity of the incorporation of ATP is slowed down, which is in agreement with the lag phase seen in the AMP incorporation.

Zone centrifugation

The results with doubly labeled ATP have shown that the RNA, which was synthesized in the presence of spermidine or high salt concentration, cannot have the same chain length. Therefore we performed zone centrifugation in a sucrose gradient of the newly synthesized RNA. The RNA was released from the enzyme-DNA-RNA complex by the addition of EDTA and sodium dodecylsulfate.

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Fig. 5 shows that the RNA synthesized in the presence of spermidine has the same chain length as the RNA of the control (low salt concentration); this confirms that more chains are initiated. On the contrary, the RNA synthesized in the presence of high salt concentration shows a remarkable shift to heavier fractions, which means that the RNA chains have to be longer.

![Figure 5. Zone centrifugation of the free RNA, which was synthesized at low salt concentration, at high salt concentration and in the presence of spermidine.](image)

**Discussion**

In our investigations we have compared the effect of salts and spermidine on the RNA synthesis catalyzed by the RNA nucleotidyl-transferase. As it is well known, both, salts and spermidine stimulate or reactivate the reaction primed by native, double-stranded DNA.

As our results indicate a certain salt concentration is necessary to hinder the precipitation of the DNA by spermidine. The optimal spermidine concentration (10 mM) leads to precipitation in low salt concentration. The favourable salt concentrations are at 40 mM (NH₄)₂SO₄ respectively 160 mM KCl.

It is unimportant for the precipitation itself, whether denatured or native DNA is present; in contrast to the stimulation of the RNA synthesis by salts or spermidine, where native DNA is required.

It is likewise unimportant for the inhibition of the RNA synthesis in the presence of high salt or spermidine concentrations, whether native or denatured DNA acts as primer. It has further been shown that a salt concentration (e.g. 320 mM KCl), which is optimal in the absence of spermidine, when added together with spermidine (10 mM) leads to inhibition.

Kinetics and zone centrifugation show the different mechanism which is the cause of the stimulation of the synthesis by spermidine and salts. Thus the experiments with doubly labeled ATP show that spermidine leads to a stimulation of the RNA synthesis by increasing the number of the chains. The zone centrifugation of the synthesized RNA has this inhibition in the presence of spermidine and high salt concentration.

Fig. 6 shows the influence of rifampicin added 5 min after the start of the RNA synthesis. We demonstrate these kinetics, because we found in this case the most striking difference. It can be seen that rifampicin leads only to a small inhibition in the control — which is in agreement with the findings of other authors — while in the presence of spermidine or high salt concentration a strong inhibition can be observed.

**Inhibition of the RNA synthesis by rifampicin**

Rifampicin inhibits the RNA synthesis by reaction with the free enzyme. We have examined this inhibition in the presence of spermidine and high salt concentration.

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proven that the single chains are not lengthened (fig. 5). Whereas in the presence of salts the initiation of the RNA chains is made more difficult\textsuperscript{13,14}. The stimulation of the synthesis must therefore depend on an elongation of the single chains. This is confirmed by zone centrifugation too (fig. 5).

The antibiotic rifampicine inhibits the free enzyme. If the antibiotic is added 5 min after the start of the RNA synthesis one finds practically no inhibition\textsuperscript{7,3}. Our experiments have shown that this only matters for low salt concentrations. In the presence of high salt concentration or of spermidine the rifampicine leads nonetheless to a clear inhibition of the RNA synthesis (fig. 6). This corresponds with the results, which our kinetics with doubly labeled ATP have given us. These kinetics have shown that after 5 min almost all chains in the control are initiated. With spermidine, however, the initiation continues, and in high salt concentration the initiation is delayed to such an extent, that after 5 min only a part of the chains have begun. Therefore rifampicine can inhibit the RNA synthesis when added 5 min after the start of the synthesis only when high salt concentrations or spermidine are present.


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Fig. 6. Kinetics of RNA synthesis with the addition of rifampicine after 5 minutes. In a total volume of 1.0 ml were incubated: 40 mM Tris buffer (pH 7.9); 4.0 mM MgCl\textsubscript{2}; 1.0 mM MnCl\textsubscript{2}; 8.0 mM 2-mercaptoethanol; 0.4 mM GTP, UTP, CTP and ATP ((8-\textsuperscript{14}C)ATP; 580 counts/min per \textmu m mole); 80 \mu g enzyme; 40 \mu g native, double-stranded DNA. Three incubation mixtures were made. The first (a) was the control, to the second (b) 240 mM KCl was added, and to the third (c) spermidine (10 mM) and (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} (40 mM) was added. Rifampicine (0.3 \textmu mole) as indicated. Aliquots of 0.1 ml of each sample were removed at the time indicated for the assay of RNA synthesis as described in the methods. a. control \textcircled{-}; with rifampicine \textbullet\textbullet\textbullet; b. 240 mM KCl \textcircled{-} \textcircled{-}; with rifampicine \texttriangle\textcircled{-} \textcircled{-}; c. spermidine \textdownarrow\textdownarrow; with rifampicine \textdownarrow\textdownarrow\textdownarrow.