The Influence of Urea and Electrolytes upon Yeast Ribonucleic Acid*

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In connection with studies of factors which influence the activity of bovine pancreatic ribonuclease, our attention was drawn to the marked stimulation of the liberation of acid soluble oligonucleotides which results when a buffered mixture of yeast ribonucleic acid and RNase is treated with high concentrations of urea. As related in another paper (1), urea at the same concentrations causes some inhibition of RNase activity as measured by other assay procedures and thus the observed stimulation appears possibly to be due to alterations in the character of RNA by urea. In view of the profound and often observed effects of electrolytes upon the properties of anionic polymers (2), we have undertaken an investigation of the influence of both salts and urea upon the structure of RNA. Our results indicate that RNA assumes a more highly cooperative structure in the presence of electrolytes, whereas treatment with urea has a markedly disrupting effect.

EXPERIMENTAL

Materials and Methods

All of the RNA used in these experiments was prepared from fresh bakers' yeast by the method of Crestfield et al. (3). Weighed samples of freeze-dried RNA were allowed to dissolve with a minimum of agitation in water or 8 M urea solution just before use. The final concentration of urea after dilution with salts and water was 3.2 M in all but the viscosity experiments; RNA is somewhat less soluble in 8 M urea than in water. All solutions were made up with double distilled water. Reagent grade urea was recrystallized from ethanol.

Absorbancy measurements in the ultraviolet were made with a Beckman model DU spectrophotometer, with the use of rectangular 1-cm. cells. Viscosity measurements were made with a conventional Ostwald viscometer at 25°. Optical rotation was visually measured with a Zeiss-Winkel polarimeter at 25° with a 2-dm. tube and the D line of sodium. Continuous titration curves were recorded during addition of 0.5 N HCl or NaOH at a constant rate to 2.5 ml. of 1 per cent RNA at 25° under a N₂ atmosphere, with the same apparatus that was used in earlier studies (4) except that a Radiometer TTT-1 pH meter (Copenhagen, Denmark) was employed in the system. The phosphorus content of RNA was determined colorimetrically after Parr bomb combustion (4).

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RESULTS

As originally reported by Crestfield et al. (3), fresh aqueous solutions of this type of RNA have a high but variable viscosity. In our experiments, the initial relative viscosity was very high and gradually decreased on standing (Fig. 1). The reduced viscosity of 1 per cent RNA was estimated by extrapolation to be about 520 cc. per gm. Initially both salt and urea lowered the viscosity but in a somewhat different manner. Addition of KCl immediately reduced the viscosity to a low value which thereafter decreased only slightly. Addition of concentrated urea, on the other hand, caused the viscosity of RNA to decrease less abruptly than did KCl although faster than in water. In the presence of both salt and urea, the viscosity fell to a low and constant value.

Additional evidence for structural differences of a typical RNA preparation in the presence or absence of salt or urea is shown in Table 1. As compared with the absorbancy of a RNA hydrolysate at 260 mμ, RNA was moderately hypochromic in aqueous solution, in 8 M urea, or in 8 M urea plus 0.08 M KCl, whereas it was strikingly hypochromic in the presence of 0.08 M KCl alone.

The specific rotation of RNA was high as compared with that of its constituent nucleotides. In the presence of 0.08 M KCl, the rotation was greatly elevated above the already considerable dextrotoration of RNA in aqueous solution. Addition of urea lowered the rotation of RNA both in the presence and absence of electrolyte. We found that the rotation of solutions of RNA in dilute KCl was essentially constant from pH 5 to 8; below pH 5, the increasing turbidity of the solutions prevented accurate measurements of the rotation. Prolonged heating at 60° at pH 6.3 in 0.08 M KCl irreversibly lowered the rotation of RNA. In the absence of added salt, a 1 per cent solution of RNA had a pH of about 7.0; upon the addition of 0.08 M KCl, the pH dropped to 6.3. As shown in Fig. 3, the entire titration curve from pH 3 to 10 was shifted toward a lower pH in 0.08 M KCl.
Influence of Urea and Electrolytes upon Yeast RNA

**FIG. 1.** The effect of aging on the relative viscosity of 1 per cent RNA in water, 0.08 M KCl, and/or 8 M urea solutions at 25°.

**TABLE I**

<table>
<thead>
<tr>
<th>Solution</th>
<th>$\eta_r$ at 1 cm.</th>
<th>$\alpha$°</th>
</tr>
</thead>
<tbody>
<tr>
<td>In water</td>
<td>196</td>
<td>+98°</td>
</tr>
<tr>
<td>In 0.08 M KCl</td>
<td>172</td>
<td>+140°</td>
</tr>
<tr>
<td>In 3.2 M urea</td>
<td>102</td>
<td>+74°</td>
</tr>
<tr>
<td>In 0.08 M KCl + 3.2 M urea</td>
<td>208</td>
<td>+118°</td>
</tr>
<tr>
<td>Alkaline hydrolysate in 0.1 M NaCl, pH 7.0</td>
<td>248</td>
<td>-13°</td>
</tr>
</tbody>
</table>

**Fig. 2.** The influence of temperature upon the specific rotation of 1 per cent RNA in 0.08 M KCl. The lower curve (O--O) depicts the rotation as measured as the temperature indicated. The upper curve (●—●) depicts the rotation of RNA at 20° after heating for not more than 6 minutes at the temperature indicated.

with particularly pronounced changes in the pH 3 to 6 and pH 9 to 10 ranges. Addition of 3.2 M urea had no appreciable effect upon the pH of the 1 per cent RNA in water either initially or after several hours and affected the titration curves only slightly. The shift of the titration curve caused by 0.08 M KCl in the added presence of 3.2 M urea was less pronounced than that in KCl alone.

**DISCUSSION**

The alteration of physical properties of RNA manifested by addition of dilute electrolytes appears to reflect structural changes which are different from those that are caused by treatment with concentrated urea. The effect of dilute KCl in lowering the viscosity of RNA is similar to the effect caused by addition of electrolytes to salt-free solutions of a number of other highly ionized linear polymers such as polyacrylic acid (5) and DNA (6-8), and appears to be due to counterion shielding of mutually repelling charged segments of the polymer chain, thereby permitting the extended structure to assume a more tightly coiled configuration. The decrease of the viscosity of aqueous solutions of RNA, especially in the presence of added urea, suggests that the nucleic acid may initially be in the form of loose aggregates which are held together by hydrogen bonds. A similar effect of urea upon the viscosity of DNA solutions has been observed (6).

Formation of even small polynucleotides from mononucleotides is accompanied by some degree of hypochromicity (9). In addition, hypochromicity appears to be related to the extent of nonrandom orientation of polynucleotide chains and is especially pronounced in multistranded structures made up of complementary or associated chains (10, 11). From our results, RNA appears to assume a more highly cooperative structure in the presence of electrolytes than in the presence of urea and electrolyte together, further implying the importance of hydrogen bonds for the secondary structure of RNA. An enhancement of the hypochromicity of DNA by dilute electrolytes has also been observed by Shaok et al. (12).

A similar interpretation may be made on the basis of the optical rotation of RNA. The high dextrorotation of RNA, especially in the presence of dilute salt, and the low rotation in the presence of urea may reflect structural changes similar to those reported by Doty and Yang (13, 14) for several polypeptides and proteins which are dextrorotatory in helical configuration but levorotatory in random coil form. Doty's recent 3

1 Presented at the New York Academy of Science Conference on "Enzymes of Polynucleotide Metabolism" on October 17, 1958, in New York City, New York.
observation that the high dextrorotation of mixtures of various complementary polyribonucleotides was reversibly decreased by "melting" the highly organized structures prompted us to determine the effect of heating upon the rotatory properties of RNA. The changes in rotation which we found on heating are similar to but are of lesser magnitude than those which he reported.

One of the most striking but least readily explained effects of dilute electrolytes upon RNA is the increase in acidity. Cox et al. have observed similar effects with DNA (15) and a bacterial RNA preparation (16). There is considerable latitude in the interpretation of RNA titration curves because the pK' ranges of constituent —NH₃⁺ and monoester phosphate groups overlap (17, 18). Inasmuch as the titration curves give no evidence for any significant number of monoester phosphate groups in this RNA preparation, an increase in the acidic properties of the amino and enol groups of the purine and pyrimidine bases appears to be responsible for the acid shift observed as the polynucleotide becomes more contracted. This decreased affinity of amino or keto groups for protons could come about by: (a) decreasing the influence of neighboring anionic phosphate groups by counterion shielding or (b) by a change in structural orientation resulting in greater spatial separation of the anionic phosphate groups from the amino and enol groups.

In contrast to the orienting effect of dilute electrolytes, treatment of RNA with 8 M urea had a markedly disrupting effect and although it decreased the viscosity, the disrupted product was relatively unoriented as judged by the lower optical rotation, the moderate hypochromicity, and the slight displacement of the titration curves. These observations suggest that, when first dissolved, RNA might exist partially in the form of aggregates or ionic micelles which slowly and spontaneously dissociate to smaller fragments. In the presence of KC1 they appear to contract to form tightly coiled structures possibly of the rotatory palate. Addition of KC1 increases somewhat its viscosity in dilute salt solution.

The conclusions reached here on the disruptive effect of urea on the structure of RNA, are in agreement with the conclusions reached in the previous paper (1), namely by breaking the hydrogen bonded structure in the substrate, urea would in effect increase the available RNA concentration; and secondly, by breaking the hydrogen bonds in the products of the enzymatic reaction, urea would also tend to solubilize some of the products of the reaction.

SUMMARY

1. The viscosity of ribonucleic acid, prepared by a detergent method, in freshly prepared aqueous solution is high but gradually decreases on standing, whereas in dilute salt solution the viscosity is low and relatively constant. Addition of high concentrations of urea increases the rate at which the viscosity of ribonucleic acid in aqueous solution is decreased and also decreases somewhat its viscosity in dilute salt solution.

2. In dilute electrolytes, the hypochromicity at 260 mλ of ribonucleic acid is greater than that in water or in 8 M urea. In concentrated urea, addition of dilute salts does not significantly affect the hypochromicity.

3. Divalent electrolyte solutions of ribonucleic acid are more dextro-rotatory than are those in water alone. Addition of urea to both decreases the dextrorotation. The alkaline hydrolysate of ribonucleic acid is slightly levorotatory. The rotation of ribonucleic acid in dilute solution is essentially constant from pH 5 to 8; it is reversibly lowered by brief heating, but irreversibly lowered if heating is prolonged.

4. Titration curves of ribonucleic acid in various solutions show that it is more strongly acidic in dilute electrolytes due to increased dissociation of weakly acidic groups in the pH 3 to 6 and pH 9 to 10 ranges.

5. These results indicate that in aqueous solution, ribonucleic acid may be at least partially in the form of aggregates of polynucleotides with an extended configuration, whereas in the presence of dilute electrolytes it assumes a more compact and highly cooperative structure. The disruptive influence of urea upon both the extended and contracted forms points to the probable importance of hydrogen bonding in the secondary structure.

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REFERENCES
