THE ACID-INDUCED AGGREGATION OF E. COLI S-RNA*

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Received May 8, 1966

Henley et al. (1966) have deduced from sedimentation and viscosity data that yeast s-RNA aggregates at low temperature. The aggregation occurs at neutral pH and at a moderately low salt concentration. In this communication, we describe a proton induced aggregation of E. coli s-RNA which takes place at room temperature. The degree of aggregation is dependent upon nucleic acid concentration. At low concentrations of s-RNA, the measured molecular weight and sedimentation coefficients indicate that the aggregates dissociate into monomer units.

METHODS AND MATERIALS

E. coli s-RNA, strain B, was purchased from General Biochemicals, Chagrin Falls, Ohio, freed of protein by mechanical shaking with phenol, dialyzed against repeated changes of 4 liters each of doubly distilled water for 72 hours and finally lyophilized. Analytical ultracentrifugation at neutral pH with both ultraviolet and schlieren optics showed only one symmetrical boundary with no detectable traces of faster or slower sedimenting species. At s-RNA concentrations above 1 mg/ml weight average molecular weights were estimated using the Yphantis (1960) multichannel technique. The expected precision of molecular weights determined in this manner is 5 to 10%. Schlieren and interference optics were interchangeably used. C_o determinations were always performed with s-RNA concentrations above 4 mg/ml

*The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval service at large. From the Bureau of Medicine and Surgery, Navy Department, Research Task MR 005.06-0001.01.

and the required C_o value for lower concentrations estimated by computation. At lower s-RNA concentrations, the Yphantis meniscus depletion technique was employed (Yphantis, 1964). Solution columns were 7 mm in length and sapphire windows were routinely used. The $\bar{V}$ used in all calculations was that of Tissières (1959). All centrifuge runs were made at temperatures of 21° to 23°. Operationally, s-RNA was dissolved in 0.3 M KCl, 0.01 KAc, and brought to the desired pH with either KOH or 10% acetic acid and then dialyzed versus buffer similarly treated. S-RNA concentration was estimated at pH 7.0 using a value of 21.4 for the specific extinction coefficient (Stephenson and Zamecnik, 1961).

RESULTS

Figure I shows the dependence of molecular weight of E. coli s-RNA upon concentration at pH 7 and at pH 4. At neutral pH, there is only a slight

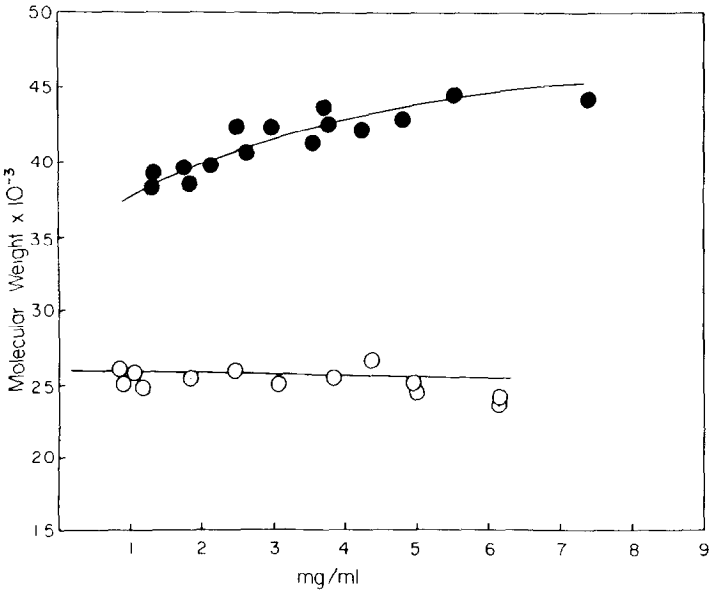

Figure I. The molecular weight concentration dependence of E. coli s-RNA at pH 7 (o) and at pH 4 (•). The solvent is 0.3 M KCl and 0.01 KAc. Rotor speeds of 12,590 rpm and 8,900 rpm were used. Equilibrium was attained in about one and one and a half hours at pH 7 and pH 4 respectively.

concentration dependence. Litt and Ingram (1964) have previously made similar observations on yeast s-RNA. Linear extrapolation to zero concentration yields a molecular weight value in the neighborhood of 26,000. At pH 4, the molecular weight is seen to vary from approximately 37,000 to 45,000 as the concentration is increased from about 1 to 7 mg/ml. Back titration from pH 4 to pH 7 resulted in molecular weight values of about 25,000 showing the process to be reversible.

The concentration dependent decrease in molecular weight at pH 4 is not due to proton scission of the polynucleotide backbone since a sample stored at 5° at pH 4 for one week showed no drop in molecular weight. Clearly, no meaningful extrapolation of the data can be made due to the nonlinear molecular weight concentration dependence. For both pH 4 and 7, data at concentrations below 1 mg/ml were erratic, possibly due to adsorption of s-RNA onto the cell surface (Yphantis, 1960).

To establish the molecular weight at lower concentrations we used the Yphantis meniscus depletion technique. Typical results are shown in Figure II

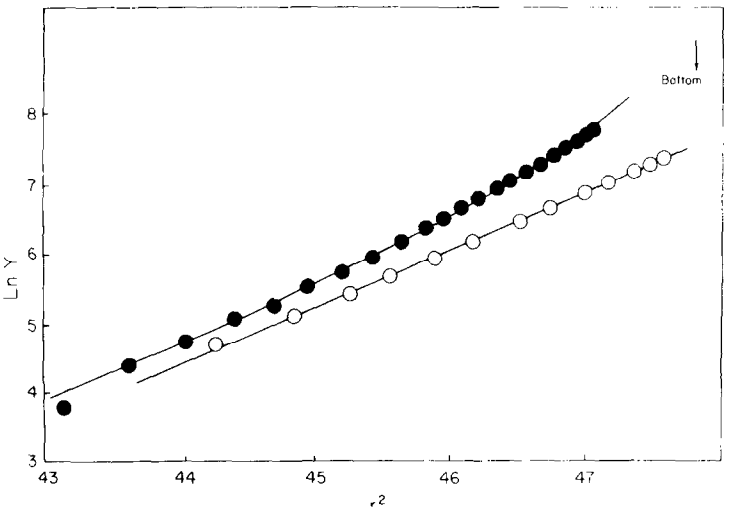

Figure II. A plot of ln Y (fringe height) versus r^2 for E. coli s-RNA at pH 7 (o) and pH 4 (•). Solvent as in Fig. I. The initial concentration at pH 4 was 0.35 mg/ml, pH 7.0 0.25 mg/ml. Rotor speed, 17,250 rpm. Final photographs taken 26 hours after speed was achieved.

The data show that at pH 7 the preparation behaves essentially as a monodisperse system. The calculated molecular weight is 26,450, in good agreement with that estimated by linear extrapolation of the data of Figure I. Clearly, at pH 4 the data reflect a polydisperse system. From the limiting slopes apparent molecular weights of 27,450 and 39,000 may be calculated. The first value clearly represents the presence of monomer units, the latter that of aggregates. In view of the curvilinearity, a higher value for the latter may be more likely since close to the bottom of the cell—i. e., at effectively higher s-RNA concentrations—the fringes were so compressed that precise determination of their position was difficult.

Clearly, the results of this experiment show that partial dissociation of the aggregates occurs and that the concentration dependence of molecular weight (below 1 mg/ml) must be quite sharp.

To determine whether the aggregates dissociated completely at very low concentration we measured sedimentation coefficients at 0.0045% with the ultraviolet optical system. The average result of 4 determinations at pH 4 was $4.0_1 \times 10^{-13}$ sec. This is to be compared with the average value at pH 7 of $3.9_8 \times 10^{-13}$ sec. The closeness of the values suggests that the aggregates do dissociate almost completely.

At a concentration of 13.7 mg/ml, a significant difference in sedimentation coefficients occurs. Thus at pH 4 and pH 7 the values are $4.7_6 \times 10^{-13}$ sec and $3.8_4 \times 10^{-13}$ sec respectively. The pH 7.0 boundary was quite symmetrical. The pH 4.0 boundary was not, showing pronounced asymmetry on the centrifugal side (Figure III). The latter point is important since the sedimentation coefficients were estimated from the rate of migration of the apparent peak position. Clearly, the sedimentation coefficient estimated by this means for the acid form of s-RNA is not a weight average value since the contribution of the higher molecular weight species is not quantitatively represented (Schachman, 1959). At extremely low concentration values this situation probably does not obtain.

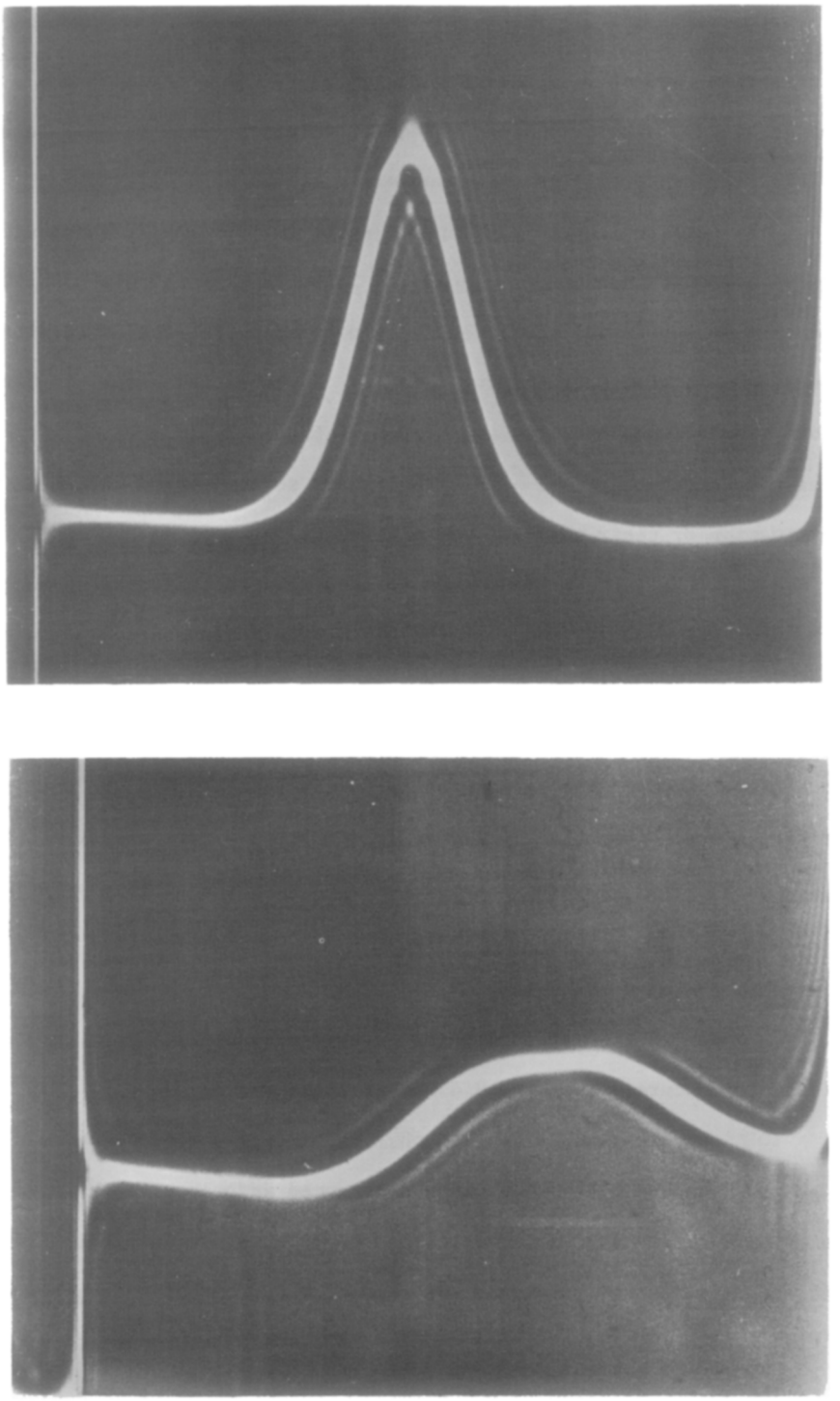

Figure III. Schlieren photos of s-RNA at pH 7.0 (top) and pH 4.0 (bottom). Solvent as in Fig. I. Photographs taken 111 and 116 minutes after reaching speed (56,100 rpm). Concentration is 13.7 mg/ml.

DISCUSSION

The results presented here show that at acid pH s-RNA molecules participate in a concentration dependent aggregation process. Assuming $\bar{V}$ to remain constant, the fact that dimer molecular weights were not achieved may indicate that some of the transfer RNA molecules comprising s-RNA are not as active towards aggregation as others. If this is so, it might be useful in fractionation procedures. Alternately, the observed molecular weight concentration dependence may be simply a reflection of the equilibrium constants governing the association.

The mechanism of the aggregation is unknown. Whether it is simply an electrostatic phenomenon resulting from the lowering of net charge by partial base protonation or if it is of a more subtle nature remains to be learned. However, in view of the results of Henley et al. (1966), it is possible that at low temperatures the proton induced aggregation reported here would exhibit a different concentration dependency.

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