THE HELIX-COIL TRANSITION OF E. COLI S-RNA AS MEASURED BY FLUORESCENCE POLARIZATION*

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Received April 29, 1966

In general, the most rewarding studies of the helix-coil transition of soluble-RNA (s-RNA) have involved the use of either sophisticated analysis of denaturation spectra (Fresco et al., 1963; Felsenfeld and Cantoni, 1964) or of optical rotatory dispersion (Lamborg et al., 1965; Lamborg and Zamecnik, 1965; Kay and Oikawa, 1966; Fasman et al., 1965).

The sensitive technique of polarization of fluorescence has only been recently applied to polynucleotide transitions (Millar and Steiner, 1965). Here, we report experiments in which the polarization of fluorescence of an acriflavine conjugate of s-RNA is measured as s-RNA is taken through the thermally induced helix-coil transition. The results are interpreted as showing that rupture of about 50% of non-covalent internucleotide bonds results in the acquisition of a great degree of freedom of internal rotation; i. e., the rotational kinetic unit of s-RNA at this temperature is equivalent to a structureless, random coil.

METHODS

S-RNA, E. coli, strain B, was purchased from General Biochemicals, Chagrin Falls, Ohio, freed of protein by repeated mechanical shaking with phenol, dialyzed 72 hours versus frequent changes of doubly distilled water

*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 lyophilized. The preparation of the acriflavine-s-RNA conjugate, measurements of polarization of fluorescence, and relaxation time calculations were performed as described in detail elsewhere (Millar and Steiner, 1965). The solvent for all measurements was 0.3 M KCl, 0.01 KAc, pH 7.0. The $S^{\circ}_{20,w}$ of the preparation was 3.9$_6$ (average of three determinations measured at temperatures of about 23°), the intrinsic viscosity 0.059 dL/g (25°), and the weight average molecular weight (determined by the Yphantis meniscus depletion method, Yphantis, 1964), 26,500. Assuming the extinction coefficient of the dye to be unaltered by conjugation there was only slightly less than one mole of dye combined with one mole of s-RNA in fair agreement with the results of Churchich (1963). A value of 21.4 was used for the specific extinction coefficient of s-RNA (Stephenson and Zamecnik, 1962).

RESULTS

Fig. I shows the polarization of fluorescence thermal profile as

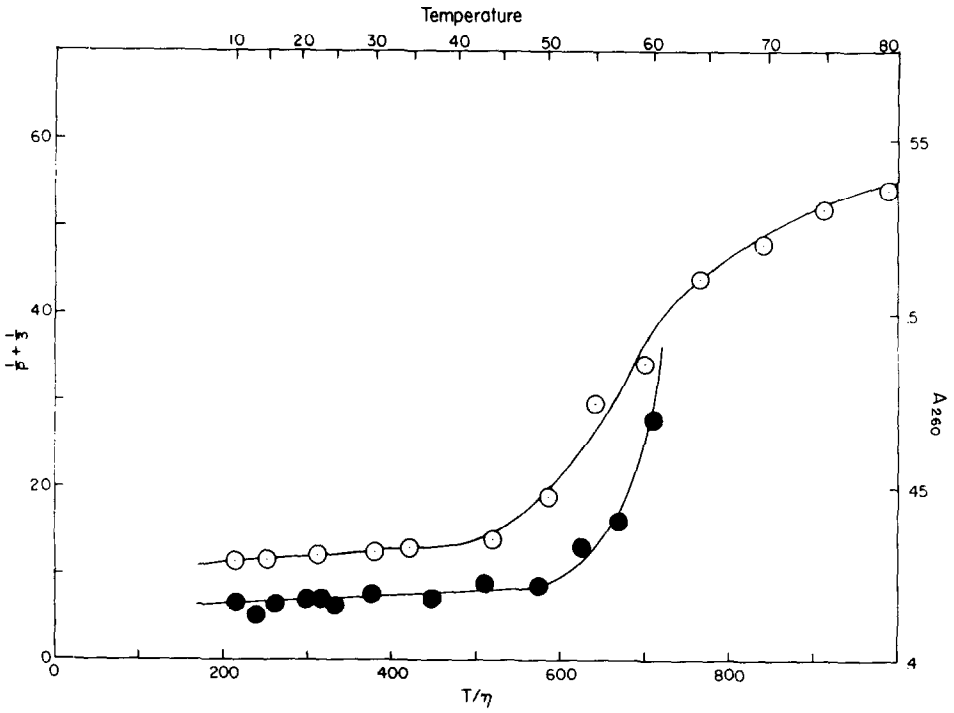

Fig. I. Polarization of fluorescence thermal profile and absorbance 260 mμ thermal profile of E. coli s-RNA. λ excitation 436 mμ, λ exit via Corning 510 mμ cutoff filter. •, 1/P + 1/3; o, A_{260}.

$\frac{1}{P} + \frac{1}{3}$ versus T/η; where T is degrees Kelvin and η is solvent viscosity. Fig. I also shows the absorbance thermal profile at 260 mμ. The temperature scale is adjusted so that T (centigrade) is in register with values of T/η. Since P (polarization) registers the presence of internal rigidity, a fall in P is expressed as a rise in $\frac{1}{P} + \frac{1}{3}$. Fig. I shows that a decrease in P begins at about 45°-50°. At 55°-60° P becomes vanishingly small. As described in detail elsewhere (Millar and Steiner, 1965) a selective gain in rotational freedom of the fluorescent label (covalently bound to the adenine residue of the single stranded CCA terminus in the present case) may heavily weight the apparent polarization. We tested for this possibility with the technique of Gottlieb and Wahl (1963), i. e. by measuring P at constant temperature and varying η by the use of various concentrations of buffered sucrose solutions. Under these conditions, if rotational freedom of the

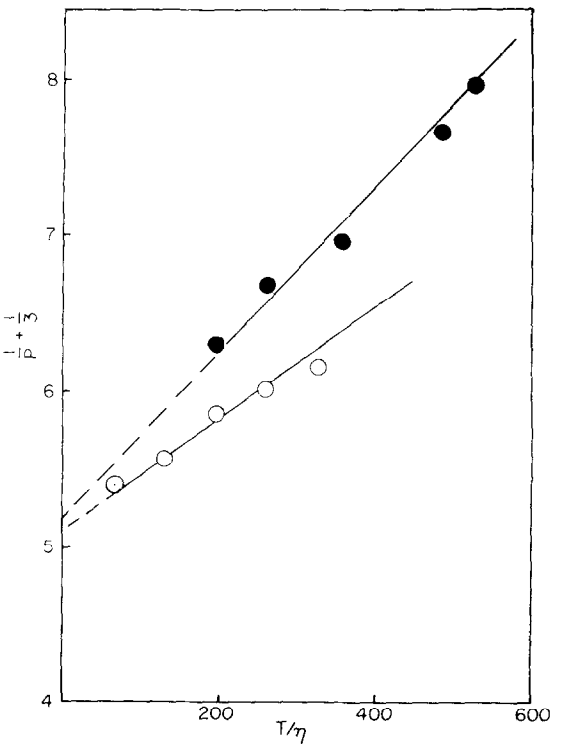

Fig. II. Polarization values at 25° and 45°. η is varied by the use of sucrose solutions. λ excitation, λ exit as in Figure I. ●, 45°; o, 25°.

label is present, $\frac{1}{P} + \frac{1}{3}$ versus T/η is at first linear but with decreasing values of T/η, $\frac{1}{P} + \frac{1}{3}$ decreases rapidly. Fig. 2 shows the result of this experiment. No evidence of curvilinearity is shown at 25° nor at 45° and the intercepts at $T/\eta = 0$ are the same within experimental error for both temperatures. This result shows that no free rotation is acquired by the label at 45°, i. e. at about the transition initiation.

DISCUSSION

The estimated T_m of the absorbance melting curve is about 57°-58° in good agreement with the results of Kay and Oikawa (1966) and Henley et al. (1966), who used only slightly different ionic strengths. That the polarization becomes vanishingly small in this temperature range indicates that the disrupted secondary forces in this region are responsible for the loss in internal rigidity. This conclusion presupposes that the label does not acquire preferential rotational freedom at 55°. One might expect, however, that as the transition begins (circa 45°) label rotational freedom would be indicated by the technique of Gottlieb and Wahl (1964). We did not find this to be so. If the loss in polarization at 55° is in part due to a fractional label rotational freedom, the idea that the structurally stabilizing forces melting in this region are responsible for the acquisition in label rotational freedom is conceivable. This could possibly happen by the loss of a conformation which restrains the rotational mobility of the label. But, evidence indicates that the CCA terminus is single stranded and not involved in the helical organization of s-RNA—i. e. always freely exposed to solvent influences—and therefore we feel that the first explanation is likely to be more correct.

One further possibility is a change in the excited lifetime (τ) of the fluorescent residue. Some but not all labelled polynucleotides (Millar and Steiner, 1965) show about a 20% to 50% decrease in fluorescent intensity from 20° to about 50°. It can be calculated (Millar and Steiner, 1965) that the loss in P shown in Fig. I cannot be accounted for by such a change in τ.

From our data, we cannot determine whether it is the melting of a group of bonds located near the terminus or a set of bonds whose loss results in a marked conformational change which is primarily responsible for the loss in internal rigidity. In this connection, Henley et al. (1966) have concluded that yeast s-RNA in 0.2 M NaCl undergoes a marked loss in asymmetry between 20° and 40°, and that above 40° yeast s-RNA behaves like randomly coiled polynucleotides. If a parallel melting mechanism between yeast and E. coli s-RNA may be assumed, then it is possible that a similar conformation event occurs with E. coli s-RNA. If it does, then the melting of a critically located set of bonds must follow the conformational change to explain the loss in rigidity we observe at 55°.

From the value of P at 25° we may calculate a value of the relaxation time (τ) of s-RNA in this solvent system and then by comparison with the value calculated for a rigid sphere of equal molecular weight compute the axial ratio of the equivalent prolate ellipsoid (Borisova et al., 1964). The axial ratio estimated in this manner is 6-7. From our sedimentation, viscosity, and molecular weight data we may compute by the Scheraga-Mandelkirn equation (1953) an apparent axial ratio of about 5-6. Thus at 25° and under our experimental conditions the translational and rotational kinetic units of s-RNA appear to be of similar size. This result indicates that at 25° the helical area immediately adjacent to the labelled terminus does not have any large rotational freedom independent of the over-all rotation of s-RNA. It is clear that this suggestion does not preclude localized conformational perturbations distant to the terminus area since they would not be detected by the polarization technique.

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