THE INTERACTION OF Hg^{2+} AND METHYL MERCURY HYDROXIDE WITH E. COLI S-RNA*

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The profound and reversible effect of the binding of Hg^{2+} upon the physical properties of DNA (Katz, 1952; Thomas, 1954; cited by Yamane and Davidson, 1961) has proved to be of a complex nature. Recently a similar effect has been observed in the RNA of tobacco mosaic virus (Katz and Santilli, 1962), as well as in several synthetic polyribonucleotides and in yeast soluble RNA (Yamane and Davidson, 1962; Kawade, 1963).

In the present report we present the results of the combination of divalent (mercuric chloride) and monovalent (methyl mercury hydroxide) mercury with E. coli S-RNA and the structural perturbations resulting from this reaction.

EXPERIMENTAL: MATERIALS

E. coli strain B S-RNA was purchased from General Biochemicals, Chagrin Falls, Ohio, and treated as described elsewhere (Millar, 1966). Methyl mercury hydroxide was a gift from Dr. Richard Simpson.

EXPERIMENTAL: METHODS

Absorption thermal profiles and optical rotation determinations were done as previously described (Millar and Steiner, 1965).

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The preparation and measurements of the polarization of fluorescence of acriflavine conjugates (the dye molecule is covalently bound to the number two or three carbon of the terminal adenosine ribose) of S-RNA were done as described elsewhere (Millar and Steiner, 1966). S-RNA AC·I and S-RNA AC·II denote two conjugate preparations. Their fluorescent and physical properties were identical.

RESULTS

Effect of mercurials on the optical rotation of S-RNA. Fig. 1 shows that Hg^{2+} sharply reduces the optical rotation of S-RNA. The decrease is approximately linear with r up to r = 1.0 at which point approximately 60% of the optical rotation has been destroyed. Methyl mercury hydroxide also reduces the optical rotation of S-RNA but much less efficiently.

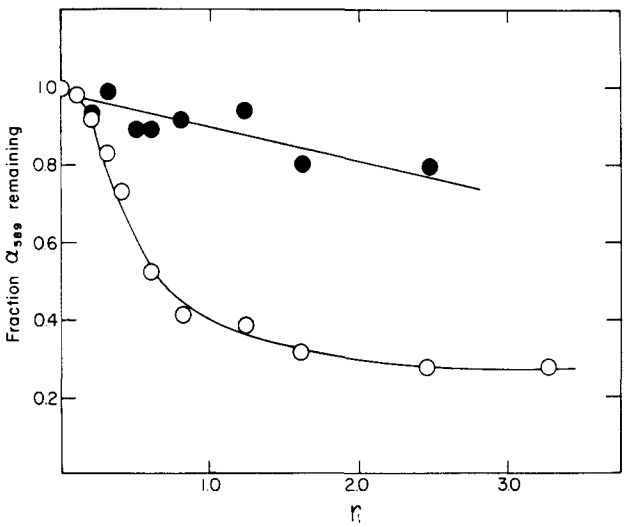

Figure 1. Effect of mercurials on the optical rotation of S-RNA. Wavelength 589 mμ. Solvent 0.01 M KAC, pH 7.0. r is the ratio of added mercurial to nucleotide calculated assuming a molecular weight of 26,000 for S-RNA.

Effect of mercurials upon the absorbance thermal profile of S-RNA. The thermal profiles of S-RNA treated with monovalent mercury tend to become less sharp than native S-RNA. However, as Fig. 2 shows, even at the highest level of monovalent mercurial studied, considerable thermolabile structure remains.

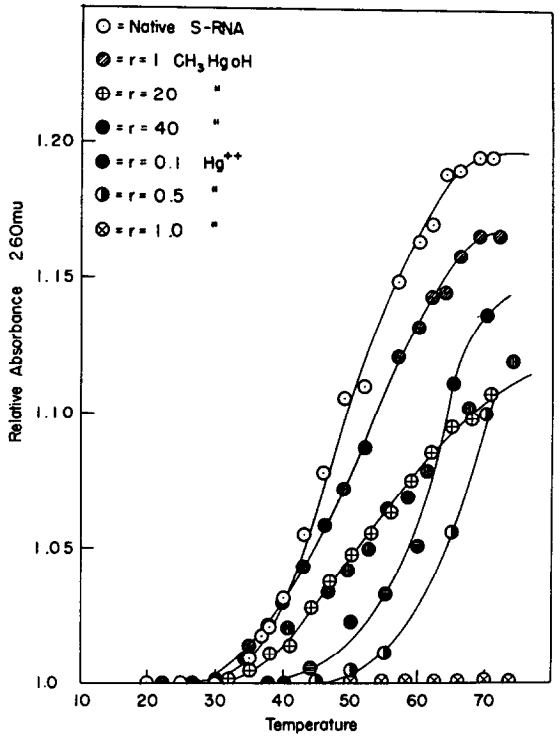

Figure 2. Effect of mercurials on the absorbance melting curve of S-RNA. Solvent as in Figure 1.

The thermal profiles of the Hg^{2+} S-RNA complexes qualitatively resemble the monovalent mercurial S-RNA complexes. Quantitatively, however, it is seen that Hg^{2+} depresses the absorbance level at any temperature at considerably lower concentrations.

Effect of mercurials on the polarization of fluorescence of acriflavine conjugates of S-RNA. The polarization of fluorescence of acriflavine conjugates of nucleic acids is highly sensitive to the state of internal rotational rigidity. It has been shown that as RNA molecules go through the helix-coil transition, the polarization (P) drops. As polarization is expressed as $\frac{1}{P} + \frac{1}{3}$, this latter quantity rises (Millar and Steiner, 1965, 1966; Millar and MacKenzie, 1966).

Fig. 3 shows that the addition of CH_3HgOH dramatically alters the

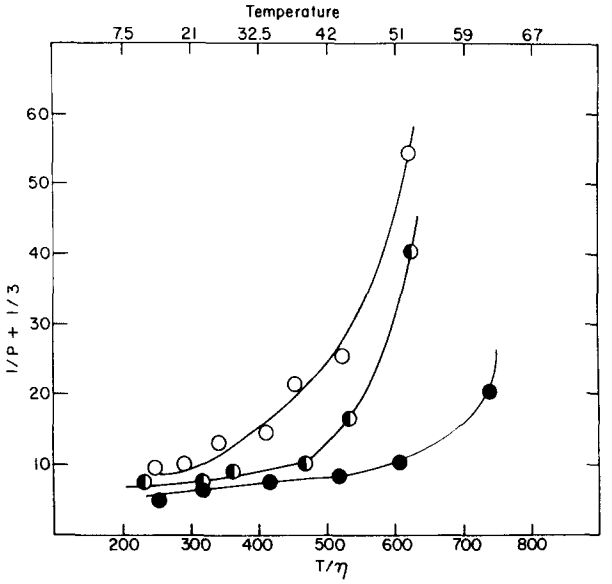

Figure 3. Effect of mercurials on the polarization of fluorescence thermal profile of S-RNA, r = 20. ◐ = native S-RNA, ● Hg^{2+} S-RNA, ○ Ch_3HgOH S-RNA. The temperature scale is adjusted to be in register with values of T/η.

polarization thermal profile's appearance with respect to native S-RNA-AC·I. The point at which P begins to decrease very sharply with small increases in temperature (which we will call the transition point) is lower than that for native S-RNA-AC·I. The addition of an equal quantity of divalent mercury to S-RNA-AC·I produces an opposite result as the transition point is shifted to higher temperatures.

Effect of mercurials upon the C-C-A terminus of S-RNA. The result of specifically increasing the rotational freedom of the labeled area of the molecule may heavily weight the apparent polarization (Millar and Steiner, 1965). To distinguish between changes in over-all internal rigidity and the effects of increased rotational freedom localized to the area of the label we determined P_0 (the value of P at infinite viscosity) by the technique of Gottlieb and Wahl (1963). The value of P_0 for S-RNA-AC·II treated with monovalent mercury was 36% lower than that for native S-RNA-AC·II, while that for S-RNA-AC·II treated with divalent mercury was 18% lower than that of the

native molecule. Results of this kind suggest a gain in rotational freedom of the label (or perhaps the adenosine terminus area) in the mercurial treated S-RNA as compared with native S-RNA (Millar and Steiner, 1965).

DISCUSSION

With the exception of guanosine, inorganic mercury is bound about ten times as tightly to free nucleosides as is methyl mercury (Simpson, 1964). This fact makes quantitative comparison between methyl mercury and Hg^{2+} difficult. However, it is possible to outline qualitatively the effects of the two reagents upon the helical structure of S-RNA.

Combination of methyl mercury with S-RNA results in changes in the absorbance thermal profiles and optical rotation which suggest alteration of the native structure. The polarization of fluorescence thermal profile suggests that at r = 20 monovalent mercurial increases the internal degrees of rotational freedom and decreases the rotational thermal stability of S-RNA (assuming no changes in the lifetime of the dye).

However, the decreased value of P_o suggests that a significant fraction of this rotational flexibility is due to the acquisition of rotational freedom by the label (and possibly the area adjacent to the label).

Studies with other nucleic acids and polynucleotides (Thomas, 1954; Yamane and Davidson, 1961; Katz and Santilli, 1962) have been interpreted in terms of bridging between nucleotides by Hg^{2+}. Clearly, in view of the dimensions of the linear complexes of divalent mercury (Katz and Santilli, 1962), the reacted polynucleotide could not be expected to keep its native helical structure. The absorbance melting curves and optical rotation results are compatible with the alteration of the native helical system by Hg^{2+}. Assuming no changes in the lifetime of the dye, the increase in rotational thermal stability of the Hg^{2+}-S-RNA complex over native S-RNA is shown by the polarization of fluorescence thermal profile of each.

These results suggest that Hg^{2+} interacting with S-RNA produces a complex with lower helical content and that the thermal stability is greater than that

of native, helical S-RNA. In spite of its apparent internal rigidity, the Hg^{2+}-S-RNA complex appears to have greater rotational freedom at the terminus area than in the native molecule.

Clearly, the alterations in secondary structure induced by mercurials suggest that the presence of free amino and imino groups are necessary to the native helical structure of S-RNA. This may indicate the involvement of these groups in intramolecular hydrogen bonding, but it is clear that native base stacking forces (Fig. 2) are also altered by combination of nucleotides with mercurials. In any event, the results presented here indicate that caution should be exercised in interpreting experiments involving the effect of mercurials (and possibly other metal ligands) on systems containing both nucleic acids and proteins, e.g. a protein synthesizing system.

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