THE INTERACTION OF MERCURIC CHLORIDE WITH RIBONUCLEIC ACIDS AND POLYRIBONUCLEOTIDES

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Mercuric ion has been shown to complex with the bases of DNA molecules resulting in a marked change in ultraviolet absorption spectra and other properties (Katz, 1952; Thomas, 1954; Yamane and Davidson, 1961). We observed that a similar interaction occurs with RNA and have been studying some properties of the complex. Recently, a number of reports have appeared on the same subject (Katz and Santilli, 1962; Yamane and Davidson, 1962), but in view of significant differences in some of the results and interpretations, we wish to report here on our own studies.

The ultraviolet spectra of various RNA's (soluble, ribosomal and tobacco mosaic virus RNA's) change in a qualitatively similar way on addition of mercuric chloride. The results on yeast soluble RNA (sRNA) are presented in Fig. 1, (a) and (b), which are the spectra in sodium and magnesium perchlorates, respectively, containing 0.01 M phosphate buffer, pH 6.5 - 7, for various values of $\underline{r}$ ($\underline{r}$ = moles of mercuric chloride added per mole of nucleotide). The results in sodium perchlorate are in harmony with those of Katz and Santilli (1962) who studied tobacco mosaic virus RNA in 0.03 M sodium acetate buffer (pH 6.38 - 6.78); that is, for $0.13 \leqq \underline{r} < 1$, there is an isosbestic point at 263 mμ, suggesting the presence of a single kind of complex in that range. As they noted, this is different from the behavior of DNA, for which two kinds of complex are found, one for $0 < \underline{r} \leqq 0.5$ and the other for $\underline{r} > 0.5$.

However, when the solvent contains magnesium ion (Fig. 1, b), the complexing of sRNA appears very similar to DNA in that it forms one kind of complex below $\underline{r} = 0.5$ (an isosbestic point at 262 mμ) and a different one above that. Ribosomal RNA in magnesium perchlorate shows an identical complexing behavior. Since RNA is generally believed to be single-stranded, these observations cast some doubt on Katz and Santilli's proposal that double- and single-stranded nucleic acids can be distinguished by their mode of interaction with mercuric ion. However, an alternate possibility that RNA molecules develop DNA-like helices extensively in the presence of magnesium ion may not entirely be excluded. This is now being investigated.

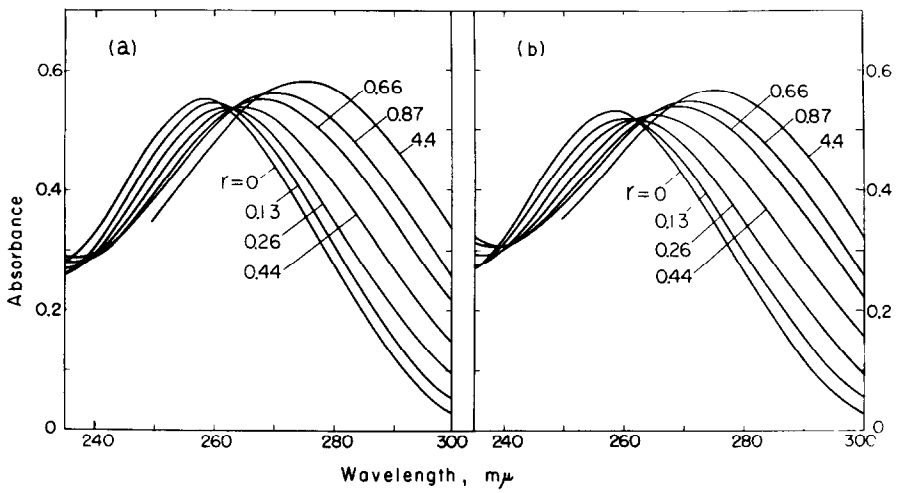

Fig. 1. The ultraviolet absorption spectra of yeast sRNA for various $\underline{r}$ (moles of $HgCl_2$ added/mole of nucleotide). (a) In 0.14 M $NaClO_4$ and (b) in 0.001 M $Mg(ClO_4)_2$, both containing 0.01 M phosphate buffer, pH 7.

To elucidate the structure of the complex, polyribonucleotides of known composition would be useful. We have studied the complexing of poly A, poly U, and their 1:1 double helix in 0.14 M sodium perchlorate at pH 6.5 - 7 (0.01 M phosphate) with the results shown in Fig. 2. The curves of poly A and poly U are in agreement with Yamane and Davidson (1962). Poly A shows the same general trend in spectral change as DNA and RNA, but, having no isosbestic point, seems to undergo less well-defined complexing. On the other hand, poly U has an isosbestic point at 276.5 mμ for $0 \leqq \underline{r} \leqq 1$, sug-

gesting the presence of a single kind of complex. Since the curves for $\underline{r}$ = 0.5, 0.76 and 1.01 coincide with each other within experimental error over the wavelength range examined, we are led to suppose that a U-Hg-U type complex is the only one to be formed below $\underline{r}$ = 1, and that its formation is essentially complete at $\underline{r}$ = 0.5. Yamane and Davidson (1962) interpreted differently, stating that the spectra of poly U represent the independent interaction of Hg ion with the uracil bases. Our interpretation is based on the assumption, which seems reasonable, that the spectrum of the Hg-U type complex, if it were formed at $\underline{r}$ = 1, must be different from that of the U-Hg-U type which is observed at $\underline{r}$ = 0.5.

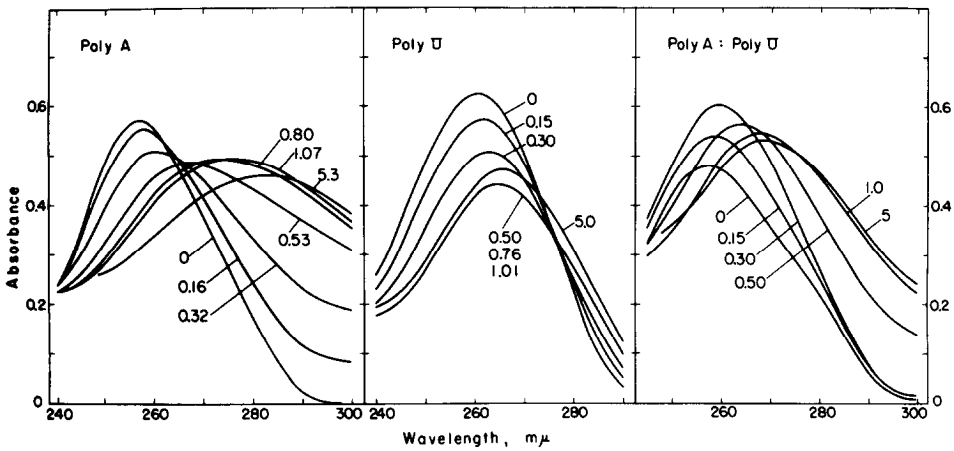

Fig. 2. The spectra of poly A, poly U, and their 1:1 complex for various values of $\underline{r}$. In 0.14 M $NaClO_4$ - 0.01 M phosphate, pH 7.

Poly A-poly U double helix is peculiar in that, on increasing $\underline{r}$, the absorption increases at first, contrary to all other nucleic acids thus far tested. (Yamane and Davidson's (1962) results are different from ours; they observed the typical behavior of two-stranded polynucleotides. This discrepancy may probably be attributed to the difference in the pH used -- 5.70 in their case and about 7 in ours.) We interpret this as indicating an extensive breakdown of the double-helix with the accompanying hyperchrom-

ism overwhelming the intrinsic decrease of absorption on complexing with Hg(II). Parallel sedimentation velocity runs substantiate this view. As shown in Table 1, while the sedimentation coefficients of poly A and poly U increase monotonously with increasing r, that of the poly A-poly U complex does not until about $r = 0.5$, above which we are probably observing a simple mixture of the separate complexes of poly A and poly U with Hg(II). The profound effect of Hg(II) on the secondary structure was also demonstrated with sRNA by the optical rotation measurements kindly performed by Prof. K. Imahori of Tokyo University.

A more detailed account of this work will be given elsewhere.

Table 1

r	Poly A	Poly U	Poly A-poly U (1:1)
0	3.8	1.1	8.6
0.15	-	6.3	8.7
0.3	7.2	8.7	5.9
0.5	10	13.5	14

Average sedimentation coefficients (s_{20}) of poly A, poly U, poly A-poly U double helix and their Hg(II) complexes. Measured in 0.14 M $NaClO_4$ - 0.01 M phosphate buffer, pH 6.5 - 7.

Acknowledgments

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References

Katz, S., J. Am. Chem. Soc. 74, 2238 (1952).

Katz, S. and Santilli, V., Biochim. Biophys. Acta 55, 621 (1962).

Thomas, C. A., J. Am. Chem. Soc. 76, 6032 (1954).

Yamane, T. and Davidson, N., J. Am. Chem. Soc. 83, 2599 (1961).

Yamane, T. and Davidson, N., Biochim. Biophys. Acta 55, 780 (1962).