

⁷ M. STAEHELIN, *Biochem. biophys. Acta* **29**, 410 (1958).

is a function of the RNA concentration, the reaction approaching completion when 0.5–1 molecule of Mg^{++} is present per molecule of RNA phosphorus. In Table I the results are therefore presented on the basis of a Mg^{++} /RNA-P ratio and show a parallelity between the formaldehyde reaction and the hypochromic effect. From a quantitative point of view it may be interesting to note that in the absence of salts the same number of formaldehyde molecules are bound as there are amino groups present and that this amount is reduced by about 65% in the presence of higher Mg^{++} concentrations. Similar values have been obtained with TMV-RNA in 10^{-2} to 10^{-1} M potassium chloride as well as with TMV-RNA prepared by means of metal containing phenol².

This strongly suggests that the amino groups of the purine and pyrimidine bases are involved in the formation of this more structured form of RNA. Mg^{++} has been shown to favour the formation of double and triple stranded helices through hydrogen bonds between base-pairing polyribonucleotides^{3,4}, probably through its effect on the negative changes of the phosphate groups. The amino groups lose their reactivity under these conditions⁷. From an analogy with these models it may be inferred that a base pairing of the purine and pyrimidine bases takes place in RNA also. If this were the case the results would indicate that about 80% of all the theoretically possible hydrogen bonds have been formed, since purines are present in about 18% excess over the pyrimidines in TMV-RNA⁸. Not all the bases, therefore, could become involved in hydrogen bonding. The unreactivity of the bases does not necessarily permit the conclusion, however, that hydrogen bonds have been formed since other ways are also conceivable by which the amino groups might be blocked. ZUBAY⁹ has recently postulated that in denatured DNA Mg^{++} might chelate with the adenine and guanine bases. But it seems unlikely that this would be the way by which Mg^{++} changes the configuration of RNA, since similar changes in configuration have also been observed by higher concentrations of monovalent ions and since under these conditions the hypochromic effect is not reversed by versene¹⁰.

Table II

Hypochromic effect in the presence of various formaldehyde and Mg^{++} concentrations (ϵ 260 m μ)

Formaldehyde concentration	H ₂ O	$3 \times 10^{-6} MgCl_2$	$10^{-5} MgCl_2$	$10^{-4} MgCl_2$
—	0.940	0.930	0.840	0.820
0.1%	0.940	0.930	0.840	0.820
0.2%	0.950	0.945	0.860	0.840
0.5%	0.980	0.970	0.900	0.875
1.0%	0.990	0.985	0.970	0.950

Yeast RNA¹¹ was incubated for 48 h at room temperature with the concentrations of formaldehyde and $MgCl_2$ indicated and the absorption at 260 m μ determined. Glass distilled water was used throughout the experiment

Interestingly in our experiments we have found no changes in the ultraviolet spectrum of ribonucleic acids upon treatment with formaldehyde in contrast to the

earlier report by FRAENKEL-CONRAT¹². The difference can be accounted for by the different concentrations of formaldehyde which were used. The effect of various concentrations of formaldehyde upon the optical properties of nucleic acid is shown in Table II. Formaldehyde in concentrations of 0.1% which earlier experiments⁷ have shown to suffice for complete reaction of all amino groups of the nucleic acid do not cause any increase in its ultraviolet absorption. An increase occurs only with higher concentrations and is especially evident in the presence of Mg^{++} .

It seems likely that at low concentrations formaldehyde reacts by forming a monomethylol derivative ($-NH-CH_2OH$) whereas at higher concentrations a dimethylol derivative ($-N=(CH_2OH)_2$) is formed which would be responsible for the spectroscopic changes. The larger increase in the presence of Mg^{++} could be an indication that formaldehyde at higher concentrations causes a reversal of the salt dependent change in configuration of RNA¹³. The absence of any spectroscopic changes at low concentrations indicates that under these conditions formaldehyde does not alter in any way the configuration of the RNA molecule. The stoichiometric relationship between the formaldehyde bound and the amino groups present as well as the evidence that under the conditions of the reaction (0.1%) only the monomethylol derivative is formed lend further support to the conclusion that in a salt free medium all amino groups are free and reactive and the reduction in formaldehyde binding by 65% upon the addition of Mg^{++} is a true expression of the number of amino groups which through hydrogen bonding or by some other means have become unreactive.

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Zusammenfassung

Die Nukleinsäure des Tabakmosaikvirus kann in zwei Formen vorliegen. In salzfreiem Milieu sind sämtliche Aminogruppen frei und reagieren mit Formaldehyd, während in Gegenwart von Mg^{++} nur etwa 35% reaktionsfähig bleiben. Diese Veränderung, die parallel mit dem hypochromen Effekt einhergeht, deutet darauf hin, dass unter dem Einfluss von Mg^{++} die Basen gegenseitige Beziehungen eingehen und dadurch dem Molekül eine geordnete räumliche Konfiguration geben.

¹² S. ZAMENHOF, G. GRIBOFF, and N. MARULLO, Biochem. biophys. Acta 13, 459 (1954).

¹³ This might explain why formaldehyde, although at 0.1% it does not react with DNA, leads to inactivation of the transforming principle of bacteria at 3%¹¹.

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Alkaloids of Apocynaceae IV¹. Dregamine, a New Alkaloid from *Voacanga dregei* E. M.

In continuation of our studies of alkaloids from different species of the family *Apocynaceae*, we have examined a

⁸ K. K. REDDI, Biochem. biophys. Acta 23, 208 (1957).

⁹ G. ZUBAY, Biochem. biophys. Acta 32, 233 (1959).

¹⁰ H. FRAENKEL-CONRAT, Personal communication.

¹¹ A. M. CRESTFIELD, K. C. SMITH, and F. W. ALLEN, J. biol. Chem. 216, 185 (1954).

¹ Alkaloids of Apocynaceae III, M. GORMAN, N. NEUSS, J. A. DEYRUP, and N. J. CONE, J. Amer. chem. Soc. (1959), in press.