A CHEMICAL METHOD FOR STUDYING THE DETAILED SECONDARY STRUCTURE OF RNA*

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Adapter RNA is the carrier of amino acids to the template for protein synthesis. Part of its function is to arrange the amino acids in a specific order prior to peptide synthesis. It does this by the following means: each adaptor combines with one--and only one--amino acid; and each adaptor is presumed to have a trinucleotide sequence which hydrogen bonds to a complementary site on the messenger RNA template. Physico-chemical measurements indicate that adapter RNA from E. coli is a single polynucleotide chain about 67 nucleotides long (Zubay, 1962; Brown and Zubay, 1960). It has been proposed that the polynucleotide chain has a bend near the middle, with the two halves of the chain interacting in double helical fashion (Brown and Zubay, 1960). In a more recent and detailed model it has been suggested that the only nucleotides not in the double helix configuration are the three in the bend, and the two on the nucleoside end which combines with the amino acid (Zubay and Bergeron, 1962). Similar structures for adapter RNA have been suggested by others (Spencer, et al., 1962; McCully and Cantoni, 1962). To test these postulated structures it would be useful to have a chemical method of isotopically labeling those nucleotides which are not in the double helix configuration.

Fraenkel-Conrat (1954) has shown that where most RNAs react with formaldehyde, DNA, under the same conditions, does not. Formaldehyde forms

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a Schiff base with the amino groups of adenine, guanine and cytosine; and the inactivity of the DNA bases is believed to be caused by steric hinderance since they are inaccessible in their native double helical configuration. Most forms of RNA also show a good deal of double helix character (Zubay and Wilkins, 1960), but their double helical regions are considerably less stable--either because they are shorter in length and/or involve less regularity in the base pairing. Hence RNA reacts with formaldehyde (Doty et al., 1959; Staehlin, 1958), but at a slower average rate than monomeric nucleotides. We thought that by applying factors known to stabilize the double helix we would find conditions under which the helical regions of RNA could be made totally unreactive to formaldehyde. In Figure 1 we see some of the results of this effort. The reaction of nucleotides and various nucleic acids with 2% formaldehyde were made in 0.1 M potassium phosphate buffer,

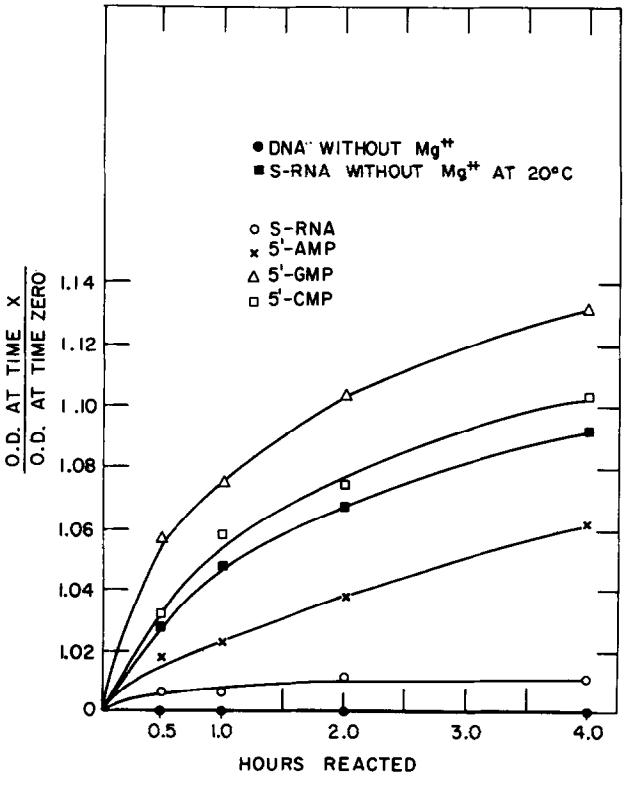

Fig. 1. Rate of increase of optical density at 260 mμ for various nucleic acids in 2% formaldehyde. Unless otherwise indicated the solvent is 0.1 M potassium phosphate buffer, pH 7.4 + 0.01 M $MgCl_2$ at 10° C. DNA and S-RNA are prepared from Escherichia coli.

pH 7.4 + 0.01 M $MgCl_2$ at 10° C unless otherwise stated. The rate of reaction was determined by measuring the increase in absorbance at 260 mμ with time. The extent of reaction is approximately proportional to the increase in absorbance. It can be seen that 5'-mononucleotides react rapidly, DNA not at all, and adapter RNA (S-RNA) only very slightly. Considerably greater rate of reaction of S-RNA occurs if the Mg^{++} is removed and/or if the temperature is raised. The protective action of Mg^{++} on adapter RNA has been observed independently by others (Penniston, 1962; Grumberg-Manago, 1962).

In Figure 2, the reactivity of adapter RNA is compared with ribosomal RNA (R-RNA) and F2 bacteriophage RNA (F2-RNA); (each with, and without 0.01 M $MgCl_2$). It can be seen that the $MgCl_2$ exerts a similar protective effect on all three nucleic acids. We conclude that these three

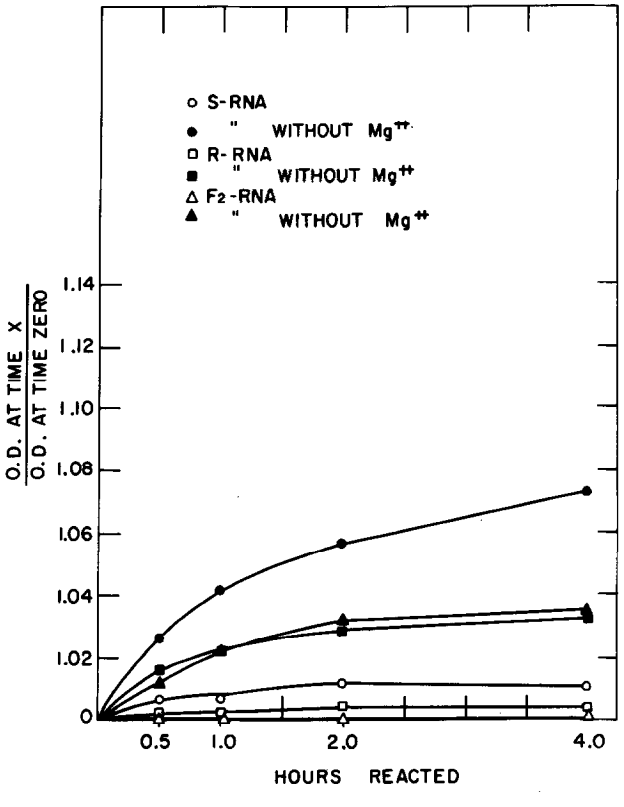

Fig. 2. Rate of increase of optical density at 260 mμ for various nucleic acids in 2% formaldehyde. All samples are dissolved in 0.1 M potassium phosphate buffer, pH 7.0 at 10° C. R-RNA = ribosomal RNA, F2-RNA = F2 bacteriophage RNA. Ribosomal RNA is prepared from Escherichia coli.

types of RNA are mostly unreactive to formaldehyde at 10° C in 0.01 M $MgCl_2$ because of steric hinderance imposed by a stabilized secondary structure.

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