QUANTITATIVE STUDIES ON THE RATE OF REACTION OF ADAPTER RNA WITH FORMALDEHYDE*

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It seems likely that the amino nucleotides within adapter RNA (S-RNA) might be classified according to their rate of reactivity with CH_2O (e.g. Zubay and Marciello, 1963), which in turn would be dependent upon their degree of involvement in folded secondary structure--in particular, internucleotide hydrogen bonding. Formaldehyde forms a Schiff base with the amino groups of adenine, guanine, and cytosine. Rate studies have been executed on S-RNA with the intent of determining the number of nucleotides in the molecule which are involved in hydrogen bonding. These studies have been carried out in the presence of 0.01 M Mg^{++} because of the stabilizing effect of divalent cation on the secondary structure (Mahler, Dutton, and Mehrotra, 1963; Nishimura and Novelli, 1963). C^{14} labelled CH_2O was used, and the specific activity of carefully washed precipitates of the RNA incubated with CH_2O was determined. Great care was necessary to prevent any loss of CH_2O in the isolation procedure, because of the instability of the Schiff base. For example, aliquots for counting were frozen dried in order to prevent a loss of about two-thirds of the activity. There is no evidence to indicate that this precaution has been observed by others.

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In Figure 1 the results of reaction with CH_2O as a function of time at different temperatures are shown. These results suggest the classification of nucleotides into three categories: those which react readily at 1° C; those which react very slowly at 1° C but readily between 10 and 26° C; and those which react readily at 37° C or above. (Although most of the CH_2O studies have been carried out on total S-RNA, preliminary indications are that specific adapter RNAs give similar results.) The number of nucleotides per RNA molecule belonging to each group is approximately 4, 8, and 39. These three groups are more discernible in Figure 2, in a similarly designed experiment in which the extent of reaction was determined as a function of temperature, using a fixed reaction time of 24 hours.

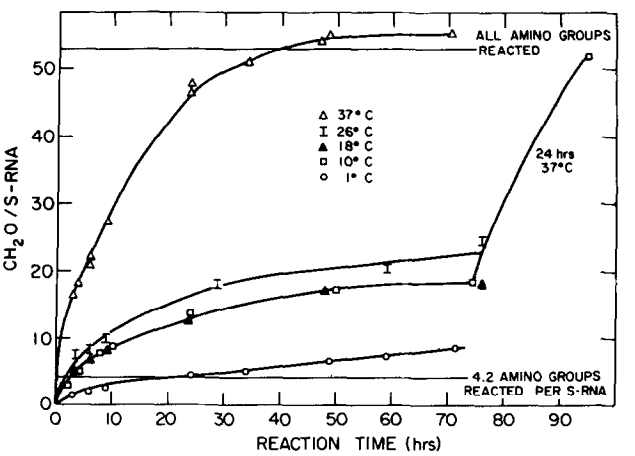

Figure 1. Number of formaldehydes reacting per S-RNA as a function of time. A 0.4% solution of S-RNA prepared from Escherichia coli in 0.1 M potassium phosphate buffer, pH 7.4 and 0.01 M $MgCl_2$ was incubated with 1.2% C^{14} labelled formaldehyde. After the desired time period an aliquot was removed from the reaction mixture, precipitated with salt and ethanol, and washed with a 1:2 mixture of 0.5 M NaCl and ethanol on a sintered glass filter. The washed precipitate was dissolved in H_2O: aliquots were taken for radioactivity and optical density measurements. The bound formaldehyde was determined from the known specific activity of the formaldehyde and the extinction coefficient for S-RNA of $E^{1\%}_{260\ m\mu} = 200$. The number of formaldehydes bound per S-RNA was computed from this, and the molecular weight of S-RNA of 24,000. Measurements were made at several different temperatures as indicated in the figure.

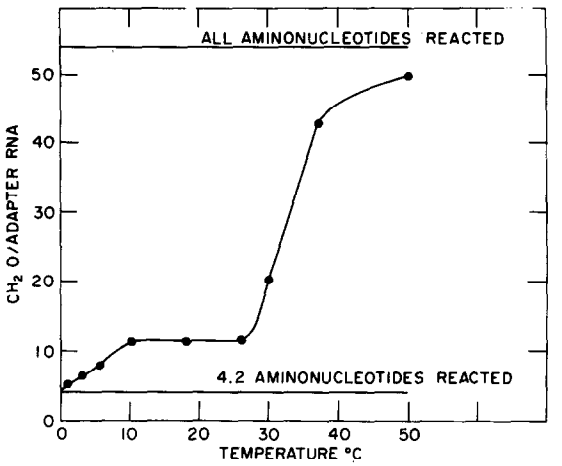

Figure 2. Number of formaldehydes reacting per S-RNA as a function of temperature. Reaction time 24 hours. Otherwise experimental conditions and procedures the same as in Figure 1.

The three groups are interpreted in the following way: the first group probably corresponds to those nucleotides which are not hydrogen bonded and not in any way sterically hindered from reacting. The second group, of intermediate activity, could be situated in a region of the RNA double helix with weak or no internucleotide hydrogen bonding. Since the amino nucleotides make up about 81% of the total (Dunn, Smith, and Spahr, 1960), 8 amino nucleotides would correspond to about 10 nucleotides or a region of 5 base pairs. The 39 bases in the third group, which is least reactive to formaldehyde, are probably situated in regions containing several hydrogen bonded base pairs. Correcting for uracil, this would involve a region of about 48 nucleotides or 24 base pairs.

A study of the hyperchromicity during reaction with CH_2O is consistent with the above interpretation. Thus at 10° C about 23% of the amino nucleotides react with CH_2O in 24 hours (see Figure 2). In spite of this, the increase in optical density at 260 mμ is only 2.2%. This rise in optical density may be considered the result of Schiff base formation and denaturation. The magnitude of these two

factors can be estimated: the increase in optical density at 260 mμ for the bases G, C, and A upon reaction with CH_2O is about 11%, 10%, and 8%, respectively. Since these bases make up 31%, 28%, and 21% of the nucleotides in S-RNA, we should expect an approximate 8% rise in optical density for 100% Schiff base formation. From the hyperchromicity of RNA on melting, it can be estimated that about 30% increase in optical density should result from complete denaturation. The reaction of 23% of the amino nucleotides at 10° C should lead to a 1.8% increase in optical density from Schiff base formation. This accounts for most of the observed rise, and suggests that very little denaturation is required for this limited reaction.

The abrupt transition in the number of nucleotides reacting with CH_2O comes at a much lower temperature (~37° C) than the melting temperature (~60° C) in this solvent. This is probably the result of two factors: 1) the cooperative breakdown of structure caused by Schiff base formation; 2) the immediate action of formaldehyde as a denaturant (CH_2O in addition to forming a Schiff base, is a denaturant).

Further studies are in progress to determine the distribution of the most reactive nucleotides in adapter RNA.

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