ON THE AMINOACYL-RNA SYNTHETASE RECOGNITION SITES OF YEAST AND E. COLI TRANSFER RNA

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The biological functions of transfer ribonucleic acids (RNA) are now considered to be: 1) the acceptance of activated amino acids from the aminoacyl-adenylate-enzyme complex, with the formation of aminoacyl-RNA, and 2) the transfer of the amino acid from aminoacyl-RNA to a polypeptide chain in association with the messenger RNA on the ribosomes. It is not known what structural specificities are involved in these two functions.

Recent studies on messenger RNA suggest that the genetic codes of amino acids may be universal (cf. Crick, 1963), which implies that the nucleotide sequences of transfer RNA from different biological species responsible for the transfer of a given activated amino acid to the ribosomes (transfer sites) may be identical, with the possible exception when degeneracy of the code is involved (Bennett et al., 1963). The present communication provides evidence suggesting that the nucleotide clusters in yeast transfer RNA, responsible for the recognition of certain aminoacyl-RNA synthetases (recognition sites), are different from those in E. coli. Furthermore the present experiments suggest that in the cases of yeast phenylalanyl- and lysyl-specific transfer RNA's, the recognition sites appear to differ from compositions of AAA and UUU respectively. These

sequences, according to the adaptor hypothesis, would be the nucleotide compositions of the RNA sites responsible for alignment of the corresponding amino acids in the proper location on the template.

In the present studies the experimental approach involves the bromination of transfer RNA. In an attempt to find a reagent which reacts with some but not all of the nucleotide constituents of ribonucleic acid, we have studied the bromination of mononucleotides and transfer RNA. It has been found that at room temperature and at a pH of 5.7 (or as low as 2.2), bromine reacts rapidly with uridylic and cytidylic, and more slowly with guanylic acid. Adenylic acid does not react with bromine under these conditions. Details of the bromination of the mononucleotides will be reported elsewhere. Similar results have been obtained in the treatment of yeast transfer RNA with bromine. The extent of bromination of the nucleotides of transfer RNA is proportional to the amount of bromine added to the reaction mixture. By choosing a low molar ratio of bromine to the nucleotide residues of transfer RNA (1:30-40), it is possible to effect the bromination of 1 cytosine and 1 uracil per 80 nucleotide residues, leaving the purine residues of transfer RNA predominantly intact. The effects of bromination on the amino acid-accepting activities of transfer RNA from yeast and also from E. coli have therefore been examined.

Transfer RNA from yeast was prepared according to the procedure of Monier et al. (1960). Twenty-four ml of various concentrations of bromine (0.025, 0.05, 0.1 and 0.2 mM) were added with stirring to an equal volume of yeast transfer RNA (0.26 mg/ml). Large volumes of dilute solutions of bromine and transfer RNA were used to avoid uneven bromination of the base residues of the population of RNA molecules. This amount of RNA contains approximately 19 μmoles of nucleotide residues. Thus, the molar ratios of bromine to the nucleotide residues of RNA are 1 to 32, 16, 8 and 4 respectively.

The reaction mixtures were allowed to stand for 10 min. at 0°C, after which they were lyophilized over night and dissolved in a volume of 1.2 ml with distilled water. Twenty µl of the variously treated transfer RNA's, each equivalent to about 100µg of original untreated RNA, were used in each of the experiments in which the effect of bromination on the amino acid-accepting activities of RNA was studied. The incorporation of C^{14}-amino acids into yeast transfer RNA was carried out as described previously (Stephenson and Zamecnik, 1961). The results are presented in Fig. 1A.

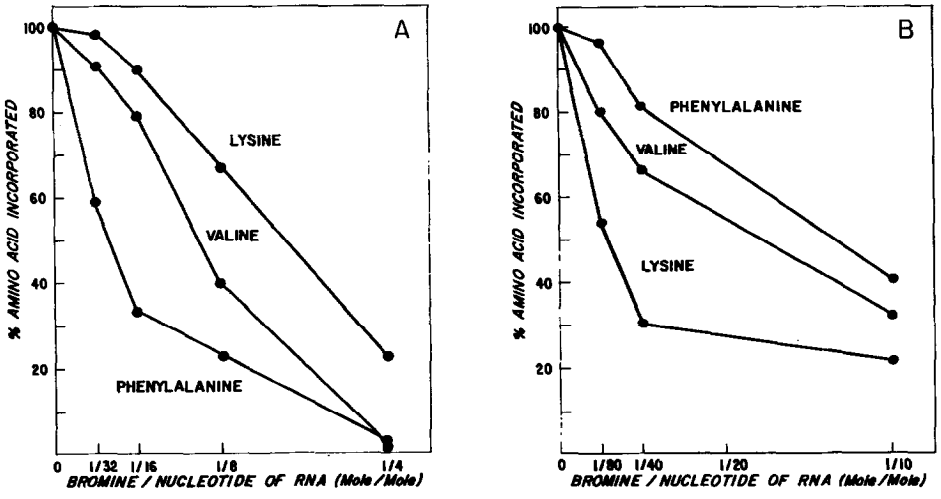

Fig. 1. The effect of bromination on the amino acid acceptor activities of transfer RNA. Each point in the figure is the average of two determinations.

A. Yeast RNA. Incubation constituents, contained in a total of 0.1 ml included: 1 µmole of Mg^{++}-ATP, 0.05 µmole of CTP, 1 µmole of phosphoenol pyruvate, 10 µg of phosphoenol pyruvate kinase, 10 µmoles of KCl, 40 µl of the ascites enzyme, 20 µl of the variously brominated transfer RNA's, 0.2 µmole of tris buffer, pH 7.5, and one of the following C^{14}-amino acids: 0.01 µmole of L-valine, 0.01 µmole of L-lysine, or 0.02 µmole of DL-phenylalanine (with specific activities of 1.69, 1.27 and 1.82 x 10^6 counts/min/µmole respectively). The reaction mixtures were incubated at 37°C for 15 min. The 100% control values for amino acid esterification are as follows, expressed as mµmoles per mg transfer RNA: phenylalanine 0.32, valine 0.49 and lysine 0.42.

B. E. coli RNA. Experimental conditions are those reported by Nathans and Lipmann (1961). The 100% control values are as follows: phenylalanine 1.4, valine 2.1 and lysine 0.97 (mµmoles per mg transfer RNA).

The percentages of the C^{14}-amino acid-accepting activities of brominated RNA are compared with those of the control samples. Among the three amino acids tested, phenylalanyl-specific transfer RNA from yeast is the most sensitive to bromine inhibition and lysyl-specific transfer RNA is the least sensitive. The accepting activities of transfer RNA for all three amino acids tested are very nearly abolished after bromination of transfer RNA at a molar ratio of 1 bromine to 2 nucleotide residues of RNA (not shown in Fig. 1A).

Transfer RNA of E. coli was purchased from General Biochemicals, Chagrin Falls, Ohio. Bromination of E. coli transfer RNA was carried out as described in the previous paragraph. In the bromination experiments, molar ratios of 1 bromine to 80, 40 and 10 nucleotide residues of E. coli RNA were used. These ratios were chosen according to preliminary experiments showing significant differences in their effects on the formation of E. coli valyl-RNA. The incorporation of C^{14}-amino acids into E. coli transfer RNA was carried out essentially as described by Nathans and Lipmann (1961). The results are presented in Fig. 1B. In contrast with yeast transfer RNA, phenylalanyl-specific transfer RNA from E. coli is consistently the least sensitive to bromine inhibition and lysyl-specific transfer RNA is the most sensitive.

The nucleotide composition and detailed secondary structure of individual aminoacyl RNA's may be unique, and bromination may possibly affect each one differently, in addition to the direct action of bromine on the site responsible for recognition of the proper aminoacyl synthetase. The following experiments were carried out to study the effect of low levels of bromination on the physical properties of yeast transfer RNA: 1) ultracentrifuge patterns of the brominated transfer RNA showed no significant difference from those of the control samples, thus providing no evidence of breakage of phosphodiester bonds of RNA following bromination. 2) Measurements of the temperature-

dependent hyperchromicity of brominated samples suggest that rupture of the secondary structure is local and limited at low levels of bromination (2-3 bromine atoms per RNA molecule). 3) The cooling curve of transfer RNA brominated at these levels retraces with fidelity the course of the heating curve, again suggesting that, to a large extent (80-95%), the secondary structure of RNA remains intact after low levels of bromination. Details of these studies of the physical properties of brominated transfer RNA will be reported later.

The results suggest to us, but do not prove, that the difference in effects of low level bromination on the acceptor activity of transfer RNA for the 3 amino acids tested reflects the result of bromination on the nucleotides of transfer RNA involved in the recognition of particular aminoacyl-RNA synthetases.

Assuming a molecular weight of 25,000, there are approximately 75-80 nucleotide residues in a transfer RNA molecule. When the transfer RNA samples from yeast are brominated with a molar ratio of 1 bromine to 32 nucleotide residues of RNA, there are, on the average, approximately 2.3-2.5 molecules of bromine for every molecule of transfer RNA. At this level of bromination there would be a maximum of 2-3 pyrimidine residues brominated per RNA molecule. It is worthy of note that at this level of bromination, the inhibitory effect on the lysine-accepting activity of yeast transfer RNA is around 2%, while the corresponding inhibitory effect with respect to phenylalanine is around 40%. The greater sensitivity of yeast phenylalanyl-RNA to bromine-induced inhibition of aminoacyl acceptance suggests the presence of a greater number (one or more) of bromine sensitive residues, probably pyrimidines, in the site for recognition of the phenylalanyl-activating enzyme. According to this reasoning, the pyrimidine contents in the recognition sites of yeast transfer RNA for the 3 amino acids tested would follow the order: phenylalanine > valine > lysine. On the other hand, the

order of the pyrimidine contents in the recognitions sites of E. coli transfer RNA is the reverse one: lysine > valine > phenylalanine. Thus, the nucleotide compositions of the recognition sites of yeast transfer RNA responsible for the acceptance of activated phenylalanine and lysine appear to differ from those of E. coli transfer RNA responsible for the acceptance of the corresponding amino acids.

The possible genetic codes for the amino acids phenylalanine, valine and lysine have been shown, respectively to be polynucleotide clusters of the following composition: UUU, UUG and AAA (Jones and Nirenberg, 1962; Wahba et al., 1963). The nucleotide clusters complementary to the genetic codes of phenylalanine, valine and lysine would be AAA, AAC and UUU respectively; and, according to the adaptor hypothesis, these sequences would be responsible for the transfer of the corresponding amino acids from transfer RNA to the template on the ribosomes. The order of pyrimidine contents of these sequences is in the reverse order for the recognition sites of the 3 amino acid-specific transfer RNA's from yeast presently studied. Thus, in the cases of yeast phenylalanyl- and lysyl-specific transfer RNA's, the recognition sites appear to differ from compositions of AAA and UUU respectively. If the nucleotide sequences of AAA and UUU do exist in the phenylalanyl- and lysyl-specific RNA's respectively,* and assume the biological function predicted by the adaptor hypothesis, one may regard the present experiments as evidence that the two biological functions of yeast transfer RNA, namely, the acceptance of the activated amino acids and the transfer of the amino acids to the ribosomes, may be represented by different structural sites.#

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* The sequence AAA has recently been found in purified yeast phenyalanyl transfer RNA in these laboratories by Dr. Peter L. Bergquist (personal communication).

We have recently been informed that studies on bromination of nucleotides and transfer RNA from E. coli, using a non-aqueous medium, are in progress in the laboratory of Professor J. Ebel (personal communication).

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