INTERACTIONS OF TRANSFER RNA FROM *E. COLI* AND METHYLATED BOVINE SERUM ALBUMIN*

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Received July 30, 1969

SUMMARY. This work indicates that free methylated albumin (MA) can form a precipitable complex with tRNA under a variety of conditions. This complex renders the tRNA reversibly inactive in accepting amino acids, non-exchangeable with free aminoacyl-tRNA in solution, and partially resistant to RNAse. The stability of the complex is partially salt-dependent and shows some ion-specificity. Urea has little effect on complex formation or dissociation. Pretreatment of the complex with pronase, followed by phenol leads to total recovery of amino acid acceptor activity of the tRNAs. Phenol treatment alone does not. No interaction between the synthetase enzymes and MA could be detected.

Columns of either kieselguhr (1) or silicic acid (2) coated with methylated albumin (MA) provide rapid methods for nucleic acid fractionation. Of the two, methylated albumin-silicic acid columns have a greater capacity and a higher resolution for the separation of the tRNAs (3). During these separations, however, loss of amino acid acceptor activity is often observed (2,4). This inactivation is generally the result of MA being eluted from the column along with the tRNA. To better understand the nature of this association, we have studied the interaction of free MA with tRNA.

MATERIALS AND METHODS. *E. coli* B tRNA was purchased from Schwarz BioResearch, and further purified by passing it through a column of Sephadex G-100 (5). MA was prepared from bovine albumin (Fraction V powder, Pentex) as described by Mandell and Hershey (1), except incubation was at 37° instead of room temperature. Measurement of radioactivity and aminoacylation of the tRNA was essentially as described earlier, but in addition contained

*Published with approval of the Director, Wyoming Experiment Station, as Journal Paper No. 392. Taken in part from a dissertation submitted by H. G. to the University of Wyoming in partial fulfillment of the requirements for the Doctor of Philosophy degree.

0.045 M NH_4Cl in the reaction mixture (5). Pancreatic ribonuclease was purchased from Worthington Biochemical Corp. and used with no further purification. Pronase (Calbiochem.) was preincubated to eliminate possible ribonuclease activity (3). L-[^{14}C]-phenylalanine (50 mCi/mmole) was purchased from Schwarz BioResearch and the mixture of L-[^{14}C]-amino acids (1 mCi/mg) from International Chemical and Nuclear Corp.

RESULTS AND DISCUSSION. The effect of increasing amounts of MA on the amino acid acceptor activity of tRNA is shown in Fig. 1. It may be seen that MA inhibits the attachment of ^{14}C-phe and a mixture of ^{14}C-amino acids to tRNA in a similar manner. This inhibition appears to be nonspecific under the aminoacylating conditions used.

If instead of measuring the amino acid accepting activity of the tRNAs in the presence of MA as shown in Fig. 1, the tubes were centrifuged (8,000 x g for 10 min.) and the absorbance of the supernatant at 260 mμ measured and plotted on the ordinate, essentially identical curves were obtained. These results suggest that the inactive tRNA-MA complex is totally precipitable. Our inability to separate the complex on either sucrose gradients or Sephadex columns supports this idea.

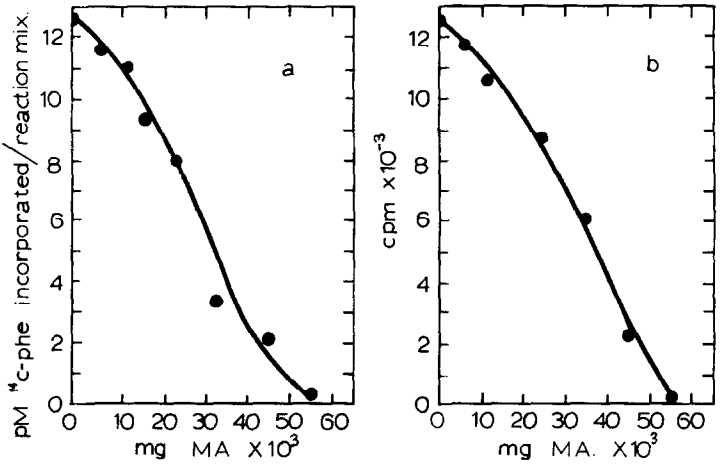

Fig. 1. Effect of MA on the amino acid acceptor activity of tRNA. The indicated amount of MA was added to each reaction mixture, containing 0.015 mg tRNA, immediately before the addition of synthetase enzymes and incubation. a. ^{14}C-phe b. ^{14}C-amino acid mixture.

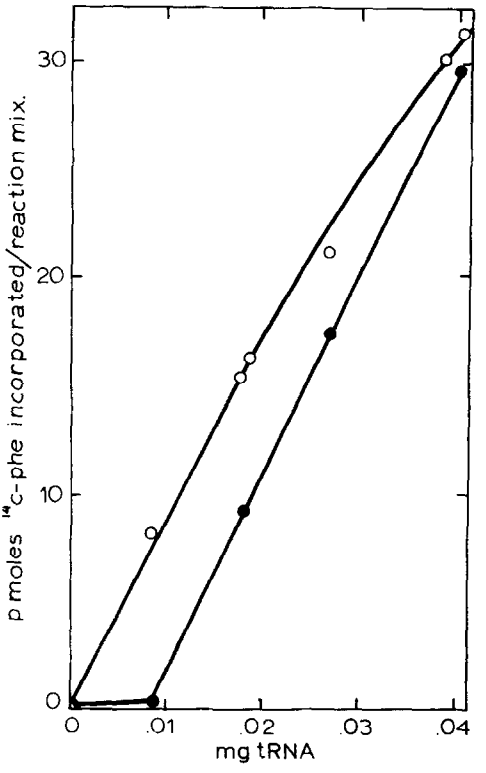

Fig. 2. Effect of tRNA concentration on phenylalanine acceptor activity. Enzyme concentration was 31 µg protein/reaction mixture. MA was added as in Fig. 1. o—o, no MA; •—•, 0.018 mg MA/tube.

When increasing amounts of tRNA are added to reaction mixtures containing constant amounts of MA, the extent of aminoacylation is linear *after* an initial inhibition (Fig. 2). MA can be considered to reduce the effective concentration of tRNA which is available for aminoacylation. The parallel lines at low tRNA concentrations suggest that exchange between bound, unlabeled tRNA and free, labeled tRNA apparently does not occur. At higher tRNA concentrations, the enzyme becomes rate limiting and causes the apparent magnitude of the inhibition to decrease.

When the tRNA concentration was held constant and increasing amounts of synthetase enzymes were added in the presence and absence of MA, the results shown in Fig. 3 were obtained. In the presence of MA the extent of aminoacylation

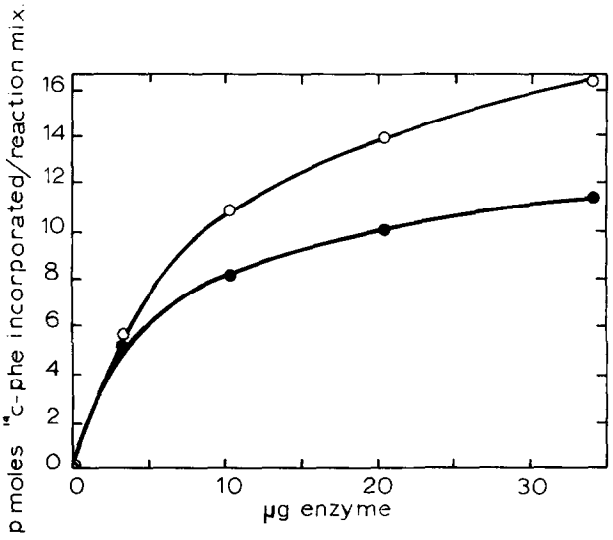

Fig. 3. Acceptance of ^{14}C-phe by tRNAs with increasing enzyme concentration. MA was added as in Fig. 1. Each reaction mixture contained 0.015 mg tRNA. o—o, no MA; ●—●, 0.014 mg MA/tube.

was less than the control. Even at enzyme concentrations 10 times greater than shown (300-400 µg), the inhibition caused by MA was not relieved.

The data shown in Figs. 2 and 3 indicate that the synthetase molecules are not affected by MA in the aminoacylation reaction mixture. All of these results suggest a complex between tRNA and MA.

The formation of a tRNA-MA complex also accounts for the increased stability of tRNA toward RNAse digestion in the presence of MA. When in the form of a complex, tRNA is hydrolyzed to acid-soluble material by RNAse at about one-third the rate of free tRNA. High molecular weight ribosomal RNA from *E. coli* was also shown to be partially protected from RNAse by MA.

When similar amounts of tRNA and MA are mixed in buffer A [0.01 M Tris-HCl, 0.01 M $MgCl_2$, 0.45 M NH_4Cl (pH 8)] and incubated at 37°, a precipitate forms. Collection of the precipitate by centrifugation and subsequent treatment with phenol does not result in extraction of the tRNA. Pretreatment of the complex with pronase, as described by Stern and Littauer (3), followed by phenol leads to essentially complete recovery of the acceptor

Table I

Amino Acid Acceptor Activity of tRNA recovered from tRNA-MA Complex

	cpm/mg tRNA	% Inhibition
tRNA control	3.7×10^5	0
tRNA control (0.02 mg) + 0.05 mg MBSA	0.90×10^5	76
tRNA*	3.5×10^5	5
tRNA* (0.02 mg) + 0.05 mg MBSA	0.85×10^5	77

*tRNA recovered from the tRNA-MA complex by pronase digestion and phenol extraction according to Stern and Littauer (3).

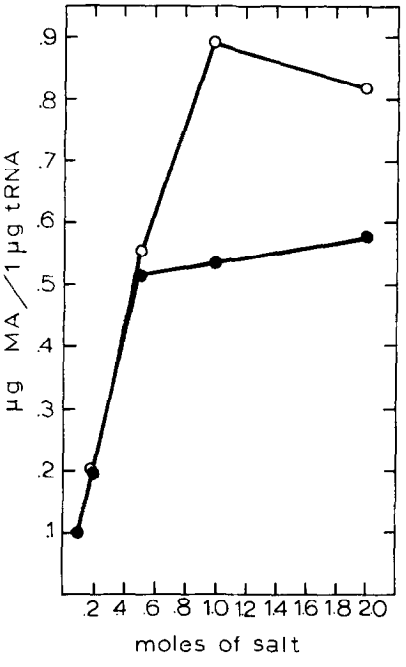

Fig. 4. The effect of cations on the amount of MA that can be added to a constant amount of tRNA without causing turbidity. o—o, KCl; ●—●, NH_4Cl.

A constant amount of tRNA (1 μg) in buffer A and increasing amounts of MA were incubated at 37° for 20 min. The absorbance at 450 mμ used as a measure of turbidity. The maximum amount of MA that would not cause turbidity was determined by plotting the amount of MA vs. turbidity and extrapolating to zero (6). Some distinction between precipitation and turbidity should be made since it was found that the tRNA-MA complex could be precipitated by centrifugation at 8,000 x g for 10 min. even when turbidity could not be detected.

activity of the tRNA (Table I). These findings indicate that the inactivation of tRNA is not the result of degradation.

The effect of salt on tRNA-MA interactions may be seen in Fig. 4. The increase of either KCl or NH_4Cl to 1M allows the tRNA solution to accommodate greater amounts of MA without causing turbidity. This suggests that interactions between tRNA and MA are at least partially ionic. The difference shown by K^+ and NH_4^+ ions might be due to the failure of the NH_4^+ ion to compete as successfully with the MA for the tRNA binding sites. The effect of increasing salt concentration on the inhibition of the amino acid acceptor activity of tRNA by MA was impossible to determine directly with the *E. coli* synthetases, since higher concentrations of NH_4Cl resulted in a partial inhibition even in the absence of MA.

The addition of up to 8 M urea, in the presence or absence of salt, only partially reduced the amount of tRNA (less than 10%) that was precipitated with MA, as compared with the control. These findings suggest that hydrogen bonding is of secondary importance in the stabilization of the protein-nucleic acid complex.

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