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POLYAMINE PREVENTION OF INHIBITION OF RAT LIVER ISOLEUCYL-tRNA FORMATION BY POLY(G), POLY(I) OR RIBOSOMES

Kazuei Igarashi, Makoto Tanaka, Kuniko Eguchi, and Seiyu Hirose

Faculty of Pharmaceutical Sciences, Chiba University, Yayoi-cho, Chiba, Japan

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SUMMARY: It is shown that rat liver isoleucyl-tRNA formation in the presence of Mg^{2+} is inhibited by poly(G), poly(I) or ribosomes and that this inhibition is prevented by polyamines. The inhibition is found to be noncompetitive with respect to tRNA.

In an E. coli cell-free system, polyamines not only increase the formation of some aminoacyl-tRNAs in the absence of added Mg^{2+} (1,2), but they also stimulate rat liver Ile-tRNA formation even in the presence of physiological concentrations of Mg^{2+} (3,4).

During the course of the studies on the mechanism of polyamine stimulation of rat liver Ile-tRNA formation, we have observed that rat liver Ile-tRNA formation in the presence of Mg^{2+} is inhibited by poly(G), poly(I) or ribosomes and that this inhibition is prevented by the addition of spermine. These findings are presented in detail in this paper.

MATERIALS AND METHOD

Materials - Ile-tRNA synthetase was purified about 130-fold from rat liver S100 fraction as described previously (4). The purified enzyme was a complex of Ile-tRNA, Gln-tRNA, Leu-tRNA, Lys-tRNA and Met-tRNA synthetases, as reported by Vennegoor and Bloemendal (5). Rat liver tRNA was prepared from S100 fraction according to the procedure of Zubay (6), except for the omission of the 2-propanol treatment and the insertion, before use, of successive dialyses of the preparation against the following buffers: 10 mM Tris-HCl (pH 7.5), 2 M NaCl and 1 mM EDTA; 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA; and 10 mM Tris-HCl (pH 7.5). E. coli Q 13 ribosomes, ribosomal subunits (30S and 50S), and rat liver ribosomes were prepared as described previously (7,8). Poly(A),

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poly(C), poly(G), poly(I), and poly(U) were purchased from Boehringer Mannheim GmbH.

Assay for Ile-tRNA formation - The standard reaction mixture (0.05 ml) contained the following: 50 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM magnesium acetate, 1 mM dithiothreitol, 2 mM ATP, 75 μg of rat liver tRNA, 0.05 μCi of [^{14}C]isoleucine (specific activity, 279 mCi/mmol), and 1.87 μg of Ile-tRNA synthetase. Polyamines were added at the concentrations indicated in the figures. After the reaction mixture was incubated at 30° C for 10 min, a 0.04-ml aliquot of each reaction mixture was placed on a paper disc (25-mm diameter) and radioactivity insoluble in cold trichloroacetic acid was counted by a liquid scintillation spectrometer.

RESULTS

Effect of synthetic polynucleotides and polyamines on Ile-tRNA formation - Since polyamines can recognize pyrimidine bases in single-stranded RNA (9-14) and bind to double-stranded RNA more strongly than to single-stranded RNA (14-16), the effect of synthetic polynucleotides on Ile-tRNA formation was studied in the presence or absence of polyamines. As shown in Fig. 1A, Ile-tRNA formation in the presence of Mg^{2+} was inhibited by poly(G) and poly(I); those were in a triple-stranded form (17). Other synthetic polynucleotides [poly(A), poly(C) and poly(U)] had essentially no effect. The addition of 2 mM spermine to the reaction mixture prevented the inhibition by poly(G) (Fig. 1B). A comparison of the effectiveness of different polyamines on the prevention of the poly(G) inhibition of Ile-tRNA formation is presented in Fig. 2. Spermidine gave an effect similar to that of spermine; however, the effective concentration was somewhat higher. Much higher concentrations of putrescine were required to prevent the inhibition of Ile-tRNA formation; even with the higher concentrations of putrescine, the prevention was not nearly as great as that accomplished by spermine or spermidine.

The formation of Leu-tRNA was inhibited slightly by poly(G) and this inhibition was prevented also by spermine (data not shown). The formation of Gln-tRNA, Lys-tRNA, and Met-tRNA with the same enzyme preparation was not inhibited by poly(G).

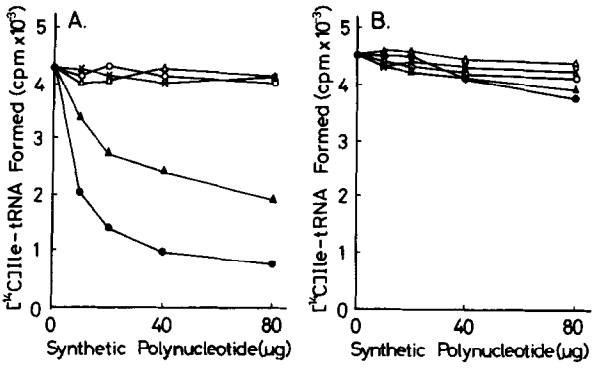

Fig. 1. Effect of synthetic polynucleotides and spermine on Ile-tRNA formation. The assays were carried out under standard conditions except that synthetic polynucleotide was added to the reaction mixture as specified. ●, poly(G); ▲, poly(I); ○, poly(U); △, poly(C); ×, poly(A). A, no spermine; B, 2 mM spermine.

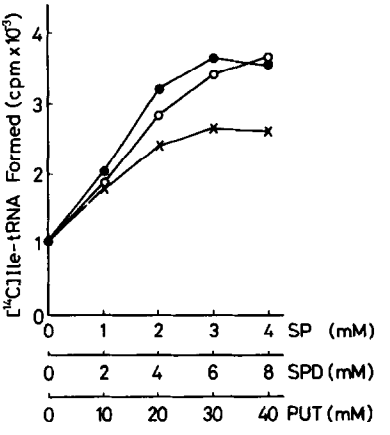

Fig. 2. Effect of polyamines on poly(G) inhibition of Ile-tRNA formation. The assays were carried out under standard conditions, except that 80 μg of poly(G) and different amounts of polyamines were added to the reaction mixture. ●, spermine; ○, spermidine; ×, putrescine.

Effect of ribosomes and spermine on Ile-tRNA formation - The effect of ribosomes on Ile-tRNA formation was then tested in the presence or absence of spermine because of the existence of double- and triple-stranded RNA in ribosomes. As shown in Fig. 3, Ile-tRNA formation in the presence of Mg^{2+} was inhibited by E. coli and rat liver ribosomes and the inhibition was prevented by

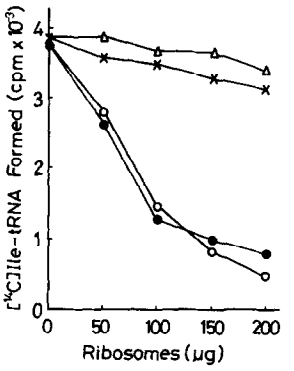

Fig. 3. Effect of ribosomes and spermine on Ile-tRNA formation. The assays were carried out under standard conditions, except that ribosomes and spermine were added to the reaction mixture as specified. ○, rat liver ribosomes; ●, E. coli ribosomes; △, rat liver ribosomes and 2 mM spermine; ×, E. coli ribosomes and 2 mM spermine.

the addition of 2 mM spermine. Since it has been reported that 30S ribosomal subunits are mainly responsible for the stimulation of polyphenylalanine synthesis by spermidine (18), the effect of E. coli ribosomal subunits on Ile-tRNA formation was examined. As shown in Fig. 4A, the inhibition by 30S ribosomal subunits was slightly greater than that by 50S ribosomal subunits. The degree of spermine prevention of the inhibition of Ile-tRNA formation by both subunits was nearly equal (Fig. 4B).

Factors which influence the inhibition of Ile-tRNA formation by poly(G) or ribosomes - The effect of tRNA concentration on the inhibition of Ile-tRNA formation by poly(G) or ribosomes was analyzed by means of double-reciprocal plots (Fig. 5). It was found that poly(G) and ribosomes were noncompetitive inhibitors with respect to tRNA. These results indicate that the poly(G) and ribosomes bind to the enzyme at a site distinct from the site binding the tRNA. The influence of Mg^{2+} and K^{+} concentrations was then tested. An increase of Mg^{2+} concentration was more effective in partially preventing the inhibition by poly(G) (Fig.6A),

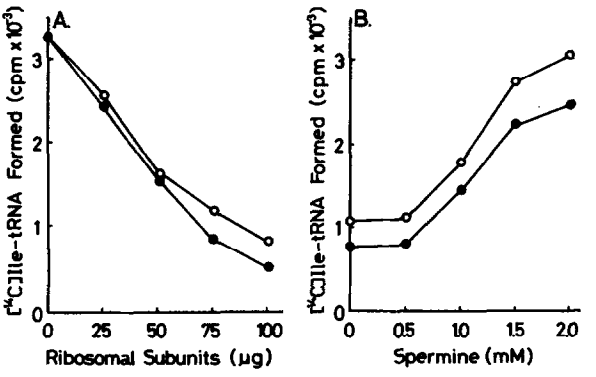

Fig. 4. Effect of E. coli ribosomal subunits and spermine on Ile-tRNA formation. The assays were carried out under standard conditions, except that the amount of ribosomal subunits was varied in (A), while in (B) the spermine was varied in the presence of 100 µg of ribosomal subunits. ●, 30S ribosomal subunits; ○, 50S ribosomal subunits.

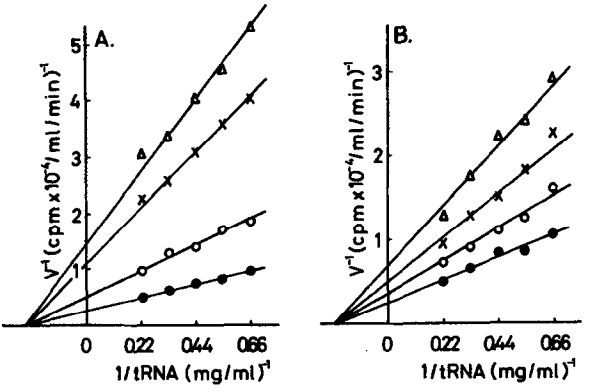

Fig. 5. Double-reciprocal plot of Ile-tRNA synthetase activity as a function of tRNA and inhibitor. Assays were carried out under standard conditions, except that the reaction mixture was incubated at 30° C for 5 min with the addition of various amounts of tRNA and poly(G) or ribosomes as specified. A. ●, no poly(G); ○, 10 µg of poly(G); X, 25 µg of poly(G); △, 40 µg of poly(G). B. ●, no E. coli ribosomes; ○, 50 µg of ribosomes; X, 100 µg of ribosomes; △, 150 µg of ribosomes.

than an increase in K^+ (Fig. 6B). However, the preventing effect of spermine was much stronger than that of Mg^{2+} and K^+.

DISCUSSION

It is of interest that ribosomes, an important factor of protein synthesis, inhibited rat liver Ile-tRNA formation in the pre-

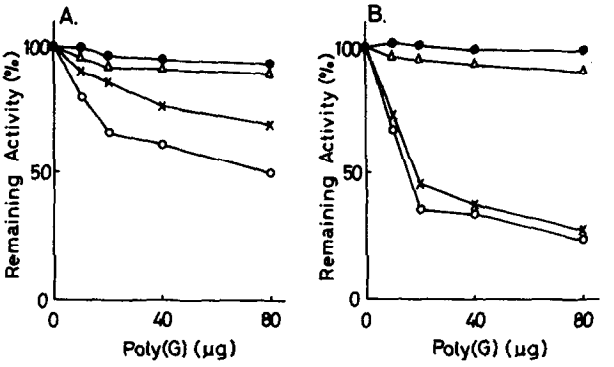

Fig. 6. Effect of Mg^{2+} and K^+ on poly(G) inhibition of Ile-tRNA formation. The assays were carried out under standard conditions, except that various concentrations of Mg^{2+} (A) or K^+ (B) were added to the reaction mixture. A. The concentration of K^+ was 200 mM. ○, 3 mM Mg^{2+} (2099); ●, 3 mM Mg^{2+} and 2 mM spermine (2807); ×, 5 mM Mg^{2+} (1742); △, 5 mM Mg^{2+} and 2 mM spermine (2515). B. The concentration of Mg^{2+} was 1 mM. ○, 100 mM K^+ (3063); ●, 100 mM K^+ and 2 mM spermine (3394); ×, 200 mM K^+ (1674); △, 200 mM K^+ and 2 mM spermine (2505). The value in parentheses is the cpm of Ile-tRNA formed without poly(G).

sence of Mg^{2+} and the inhibition was prevented by polyamines. These results, together with the previous finding that Ile-tRNA formation in the presence of Mg^{2+} was stimulated by the addition of polyamines (3,4), suggest that polyamines may play an important role in protein synthesis by regulating aminoacyl-tRNA formation.

Preliminary studies in our laboratory on the effect of spermine on Ile-tRNA formation by partially purified Ile-tRNA synthetase from various sources (E. coli, Bacillus thuringiensis and wheat germ) revealed that only wheat germ Ile-tRNA formation is stimulated by polyamines. This suggests that Ile-tRNA formation by eucaryotic cells is controlled by polyamines. Experiments are in progress to elucidate the mechanism of polyamine stimulation of eucaryotic Ile-tRNA formation.

Experimental data from our laboratory have indicated a discrepancy in the effect of polyamines on the formation of Leu-tRNA by rat liver preparations, as a crude enzyme preparation was stim-

ulated by polyamines (3) while a purified enzyme preparation was not stimulated (4). This difference could be explained on the bases of a contamination of the crude enzyme preparation with double- or triple-stranded RNA and the consequential prevention by polyamines of the inhibition of Leu-tRNA formation by the contaminated RNA.

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REFERENCES

1. Takeda, Y. and Igarashi, K. (1969) Biochem. Biophys. Res. Commun. 37, 917-924.
2. Igarashi, K., Matsuzaki, K. and Takeda, Y. (1972) Biochim. Biophys. Acta, 262, 476-487.
3. Igarashi, K., Takahashi, K. and Hirose, S. (1974) Biochem. Biophys. Res. Commun. 60, 234-240.
4. Igarashi, K., Eguchi, K., Tanaka, M. and Hirose, S. (1978) Eur. J. Biochem. 82, 301-307.
5. Vennegoor, C. and Bloemendal, H. (1972) Eur. J. Biochem. 26, 462-473.
6. Zubay, G. (1962) J. Mol. Biol. 4, 347-356.
7. Igarashi, K. and Kaji, A. (1969) Proc. Natl. Acad. Sci. U. S. 62, 498-505.
8. Igarashi, K., Hikami, K., Sugawara, K. and Hirose S. (1973) Biochim. Biophys. Acta, 299, 325-330.
9. Levy, C. C., Mitch, W. E. and Schmukler, M. (1973) J. Biol. Chem. 248, 5712-5719.
10. Schmukler, M., Jewett, P. B. and Levy, C. C. (1975) J. Biol. Chem. 250, 2206-2212.
11. Igarashi, K., Watanabe, Y. and Hirose, S. (1975) Biochem. Biophys. Res. Commun. 67, 407-413.
12. Igarashi, K., Kumagai, H., Watanabe, Y., Toyoda, N. and Hirose, S. (1975) Biochem. Biophys. Res. Commun. 67, 1070-1077.
13. Yanagawa, H., Ogawa, Y. and Egami, F. (1976) J. Biochem. (Tokyo) 80, 891-893.
14. Igarashi, K., Aoki, Y. and Hirose, S. (1977) J. Biochem. (Tokyo) 81, 1091-1096.
15. Ikemura, T. (1969) Biochim. Biophys. Acta, 195, 389-395.
16. Stevens, L. and Pascoe, G. (1972) Biochem. J. 128, 279-289.
17. Rich, A. (1958) Biochim. Biophys. Acta, 29, 502-509.
18. Igarashi, K., Yabuki, M., Yoshioka, Y., Eguchi, K. and Hirose, S. (1977) Biochem. Biophys. Res. Commun. 75, 163-171.