SITE OF INHIBITION OF YEAST LYSYL tRNA SYNTHETASE BY POLYURIDYLIC ACID

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In the presence of poly U up to 90% inhibition of charging of lysyl-tRNA by purified lysyl-tRNA-synthetase could be obtained. No inhibition of enzyme-AMP-lysine complex formation could be demonstrated nor was there any inhibition of pyrophosphate exchange. The enzyme-AMP-(^{14}C)lysine complex was isolated by sucrose density gradient centrifugation and a 75% inhibition by poly U of transfer of (^{14}C) lysine to tRNA was obtained. An enzyme-AMP-lysine-poly U complex was isolated and at low levels of tRNA no transfer occurred.

Various workers have reported the inhibition of the aminoacylation of tRNA by synthetic polynucleotides and investigations in this laboratory (1) had shown that polyuridylic acid would inhibit the charging of yeast tRNA by purified yeast lysyl tRNA synthetase and that this inhibition was competitive. The effect of poly U on other aminoacyl synthetases of yeast was examined and no inhibition occurred with the exception of the valine tRNA synthetase. Studies on the inhibition of the lysine system by poly U were continued in order to determine the site of the inhibition and are the subject of this report.

Methods

Enzyme preparation :

Lysyl-tRNA-synthetase was partially purified from strain H4 of Saccharomyces cerevisiae, as described previously (1).

Lysyl-tRNA formation :

Standard reaction mixture contained : 10 μmoles Tris, 1 μmole $MgCl_2$, 0.2 μmoles ATP, 0.1 μC (^{14}C) lysine (133.8 μC/μmole), 2.5 A_{260} units yeast tRNA, and protein as indicated,

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in a final volume of 0.1 ml, pH 8. After incubation at the indicated time and temperature, 50 µl samples were assayed for (^{14}C)-lysyl tRNA as previously described.(1) Radioactivity was measured by means of a Packard Tri-Carb liquid scintillation counting system, Model 314 EX.

Enzyme-AMP-lysine complex formation :

The standard incubation mixture was as described above with the addition of 50 µg of bovine serum albumin. No tRNA was added. The amount of complex formed was determined either by filtration through Millipore and measuring the amount of (^{14}C) lysine retained, or by measuring the amount of transfer of (^{14}C) lysine to tRNA, as described above.

ATP-(^{32}PP) exchange reaction :

Incubation mixture contained : 10 µmoles Tris, 1 µmole $MgCl_2$, 1 µmole NaF, 0.2 µmole ATP, 0.23 µmoles sodium pyrophosphate (^{32}P) (3500 cpm/nmole), 1 µmole lysine, and 0.55 µg protein in a final volume of 0.1 ml, pH 8. Appropriate blanks were run in the absence of lysine. After 90 sec incubation at the indicated temperature the reaction was stopped by the addition of 0.5 ml of 0.02M sodium pyrophosphate in 7% perchloric acid. 2 mg of acid-washed Norit A was added, samples were mixed well and the charcoal was collected by filtration on Whatman GF/C glass filters which had been washed with 0.04M sodium pyrophosphate. Samples were washed with 0.04M sodium pyrophosphate, dried and counted in 5 ml of toluene scintillant.

Sucrose density gradient centrifugation :

Gradients of 5-20% sucrose were prepared in 0.05 M succinate (0.05M KCl, 0.001M EDTA, 0.01M mercaptoethanol), pH 6. Centrifugation was carried out in a Backman L2-65 centrifuge at 3°C in a SW65 rotor for the time and at the speed indicated.

Alcohol dehydrogenase was employed as standard and was assayed as described by Letendre et al. (1).

Materials

Polyuridylic acid was prepared as described by Grunberg Manago et al. (2) with E. coli polynucleotide phosphorylase. Yeast tRNA was purchased from Boehringer (Germany) and used without further purification. (^{14}C)lysine and (^{32}P)sodium pyrophosphate were obtained from C.E.A. (Saclay, France).

Results

Effect of poly U on the formation of lysyl-tRNA :

An inhibition of 80-85% was obtained in the presence of 0.01 A_{260} units of poly U, and increasing the poly U concentration increased the inhibition only slightly. As shown in Table I it was possible to obtain a 91% inhibition of the overall charging reaction in the presence of 0.1 A_{260} units of poly U. When the enzyme was preincubated for 2 min in the presence of lysine and ATP to allow formation of the enzyme-AMP-lysine complex prior to the addition of poly U, the inhibition remained at 95%. The lower level of charging in the preincubated control sample is undoubtedly a reflection of the instability of the enzyme and it has been shown that the presence of tRNA will protect the enzyme against inactivation by dilution. The results suggested that, since the same level of inhibition was obtained in the preincubated sample when the enzyme-AMP-lysine complex was already formed, the inhibition by poly U was at the second step of the reaction, that of transfer of (^{14}C)lysine to tRNA. In order to further elucidate the process, the individual steps of the reaction were examined.

TABLE I

Inhibition of lysine charging by poly U

Sample	No preincubation		Preincubation	
	*Charging	% Inhibition	*Charging	% Inhibition
Control	80		43.6	
+ Poly U (0.1 A_{260})	7	91	2	95

*pmoles lysyl-tRNA formed

0.55 μg protein was added to the standard incubation mixture. All tubes were incubated for 2 min at 10° and 50 μl aliquots were removed and tested as described in Methods. In the preincubation samples the enzyme was incubated in the presence of ATP and lysine for 2 min at 10° and poly U and tRNA were added at zero time.

Effect of poly U on ATP-(^{32}PP) exchange :

Table II shows the result obtained when increasing concentrations of poly U were added to the ATP-(^{32}PP) reaction mixture. Although 0.01 A_{260} units inhibit the overall reaction by

80%, no inhibition of the ATP-(^{32}PP) exchange can be detected, even at a 10-fold concentration of poly U.

Effect of poly U on enzyme-AMP-lysine complex formation :

Enzyme-AMP-lysine complex was formed as described in

TABLE II

Lysine-dependent ATP-(^{32}PP)exchange in presence and absence of poly U

Sample	Poly U added (A_{260})	*Exchange	
		10°	25°
1	-	0.516	1.48
2	0.01	0.503	-
3	0.05	0.548	1.43
4	0.10	0.547	1.68

* nmoles of (^{32}PP) exchanged

Standard incubation mixtures were prepared with the indicated concentrations of poly U. After 90 sec incubation the reaction was stopped and the samples assayed as described in Methods.

TABLE III

Enzyme-AMP-lysine complex formation in presence and absence of poly U

Sample	Poly U added (A_{260})	* Complex formed	
		10°	20°
1	-	22.2	16.4
2	0.05	24.2	18.7
3	0.1	29.7	20.1
4	0.9	24.4	20.1

* pmoles of (^{14}C) lysine bound

11 μg of protein was added to the standard reaction mixture described in Methods with the indicated amounts of poly U. After 2 min incubation at the designated temperatures, the tubes were chilled and 75 μl samples were filtered through Millipores which had been washed with cold 0.1M Tris-HCl, pH 8. Filters were washed with 10 ml of the same solution in 2 ml portions, dried and counted in 5 ml of toluene scintillant.

Methods and was isolated either by sucrose density centrifugation or by a modification of the Millipore technique of Yarus and Berg (3,4). Due to the instability of the complex, the efficiency of the recovery varied, but within a set of experiments, under the same conditions and the same lot of Millipore, the results were reproducible. The results of an experiment in which the complex was formed in the presence of increasing levels of poly U are shown in Table III. Controls were run under the same conditions to determine non-specific binding to BSA in the absence of enzyme and the results shown are the corrected values. No binding was detected in the absence of ATP. As can be seen from the Table, no inhibition of complex formation occurred in the presence of poly U.

Effect of poly U on transfer of (^{14}C) lysine to tRNA :

Formation of the enzyme-AMP-(^{14}C) lysine complex was carried out as described in Methods and the complex was isolated by sucrose density gradient centrifugation. After 5 hours at 65,000 rpm, fractions were collected and tested for charging activity and for their ability to transfer (^{14}C) lysine in the presence and absence of poly U. Table IV shows the results of an experiment in which two different concentrations of tRNA were

TABLE IV

Effect of poly U on the transfer of (^{14}C) lysine from the enzyme-AMP-lysine complex to tRNA

Concentration of tRNA (A_{260})	Poly U added (A_{260})	*Transfer	% Inhibition
2.5	-	3.7	-
	0.02	2.4	35
0.1	-	3.17	-
	0.02	0.8	75

* pmoles of (^{14}C) lysyl-tRNA formed

Complex was isolated on sucrose gradient as described. 75 µl samples were tested for transfer of (^{14}C) lysine to tRNA in the presence and absence of poly U. Incubation mixture was as described for the formation of lysyl-tRNA except that (^{14}C)lysine was not added.

employed. As the results indicate, poly U inhibits the transfer of lysine from the complex and this inhibition increases with lower levels of tRNA where there would be less displacement of the poly U (1). It was also possible to isolate an enzyme-AMP-lysine-Poly U complex with which at low levels of tRNA, transfer was completely inhibited. Fig. 1(a) shows the normal profile of of the enzyme-AMP-lysine complex formed in the absence of poly U and isolated by sucrose density gradient centrifugation. The enzyme-AMP-lysine-Poly U complex is shown in Fig. 1(b). As shown, there is a shift in the peak indicating complex formation with poly U. Only at the higher concentration of RNA could any transfer of lysine to tRNA be detected.

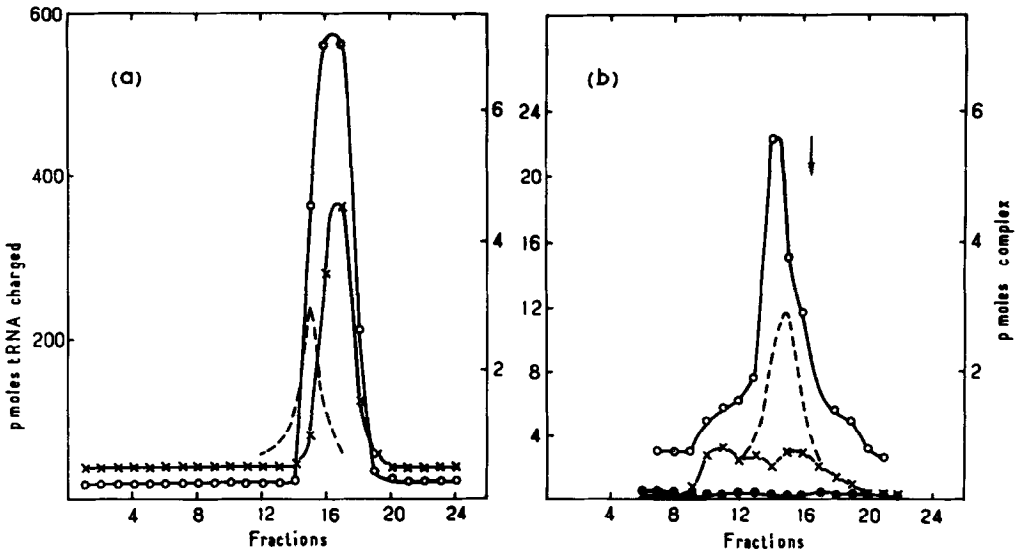

Figure 1

Isolation of enzyme-AMP-lysine and enzyme-AMP-lysine-poly U complexes on sucrose gradient

Complexes were formed as described in Methods and layered on a 5-20% sucrose gradient after the addition of 1 μmole of EDTA. 11 μg of enzyme protein was employed.

(a) normal complex
(b) complex formed in the presence of poly U (1.8 A_{260})

– – – – ADH activity
o——o enzyme activity
x——x (^{14}C) lysine transferred (tRNA, 2.5 A_{260})
o——o (^{14}C) lysine transferred (tRNA, 0.1 A_{260})

Discussion

The results presented in this report clearly indicate that the site of inhibition by poly U is the second step of the reaction, that of transfer of lysine to tRNA. This is not an unexpected finding since it was shown (1) that poly U was a competitive inhibitor of tRNA, was bound to the enzyme, and could be displaced by tRNA. (The complex formed was shown to be specifically with $tRNA^{lys}$ and these results will be discussed in a following publication.) It is probable, therefore, that the two polymers bind at the same site.

The identification of the recognition site on tRNA which enables it to be recognized by its specific synthetase is a problem yet to be solved. Several approaches have been employed and it appears that the requirement differs with each tRNA. Imura et al. (5) suggest that in the case of yeast alanyl-tRNA the recognition site involves specifically residues 5,6, and 7 from the acceptor end of the molecule. The terminal adenosine was found to play a major role in the binding of E. coli serine tRNA to the enzyme (6); however, Yaniv and Gros (7) found that removal of the terminal adenosine did not prevent the binding of E. coli valine tRNA. Studies by Roy and Tener (8), using several chemically modified tRNA's also indicated differences between the individual requirements.

In the case of the yeast lysyl synthetase the inhibition by poly U appears to be specific in that, with the exception of the valine system, none of the other synthetases were inhibited, nor were any other homopolymers tested inhibitory. Poly U and $tRNA^{lys}$ appear to bind at the same site on the enzyme and one is led to consider the possibility of a sequence of U's in the tRNA which is mimicked by the poly U , although the possibility of a particular conformation of the poly U cannot be disregarded. Investigations are currently under way in this laboratory in an attempt to clarify this problem.

References

1. C. Letendre, J. M. Humphreys and M. Grunberg-Manago, Biochim. Biophys. Acta, 186 (1969) 46.
2. M. Grunberg-Manago, P. J. Ortiz and S. Ochoa, Biochim. Biophys. Acta, 20 (1956) 1969.
3. M. Yarus and P. Berg, J. Mol. Biol., 28 (1969) 479.
4. S. A. Berry and M. Grunberg-Manago (submitted to Biochim. Biophys. Acta)

5. N. Imura, G. B. Weiss and R. W. Chambers, Nature, 222 (1969) 1147
6. M. H. Makman and G. L. Cantoni, Biochemistry, 5 (1966) 2246
7. M. Yaniv and F. Gros, J. Mol. Biol., 44 (1969) 17
8. K. L. Roy and G. M. Tener, Biochemistry, 6 (1967) 2897