REVERSIBLE MODIFICATION OF LYSYL-tRNA WHICH AFFECTS ITS BINDING TO POLY A-RIBOSOME COMPLEXES

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INTRODUCTION

The functions of transfer RNA (tRNA) include accepting amino acids, and transferring the amino acids to a specific site in the messenger RNA-ribosome complex (Berg, 1961). To date the evidence for the necessity of a specific conformation of the tRNA for these functions has been limited to two types of results. Carbon et al. (1965) showed that mild oxidation of thiopyrimidine-containing Escherichia coli tRNA prevented it from accepting amino acids. Peterkofsky (1964) demonstrated with a heterologous system that methyl deficient E. coli tRNA had a decreased capacity to be saturated with yeast activating enzyme.

The present results indicate that the conformation of lysyl-tRNA from Bacillus subtilis can be altered by mild iodine oxidation. The oxidized lysyl-tRNA has an altered elution pattern from a methylated albumin kieselguhr (MAK) column and has a decreased binding capacity to a poly A-ribosome complex. The oxidized tRNA can be reactivated for the binding function by treatment with thiosulfate. To our knowledge this is the first demonstration of a requirement for a specific conformation of tRNA in the binding or translation process.

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MATERIALS AND METHODS

Organisms and medium: B. subtilis W23 was used as the source of tRNA and aminoacyl synthetase. Penassay medium (Difco) was used for growing cells. Log phase cells were harvested at a density of 1-2 x 10^8 cells per ml. E. coli Q13, kindly provided by Dr. L. Overby, was used for the preparation of ribosomes. These cells were grown to mid-log phase in Penassay medium (Difco) at 37 C.

Preparation of tRNA: The tRNA was extracted from cells as described by von Ehrenstein and Lipmann (1961), except for three additional phenol extractions. The tRNA was treated with 0.5 M tris-HCl buffer, pH 8.8, for 60 min at 35 C to remove bound amino acids. The solution was made 1.0 M in NaCl and the tRNA was precipitated by the addition of 2 volumes of ethanol. The precipitate was dissolved in 0.01 M Tris-HCl buffer, pH 8.0 and dialysed against the same buffer overnight. After concentrating by lyophilization, the tRNA was stored in the frozen state.

Preparation of aminoacyl-tRNA synthetase: The synthetase was prepared generally as described by Zubay (1962).

Preparation of aminoacyl-tRNA: The reaction mixture of 0.5 ml contained in μmoles: Tris-HCl buffer, pH 7.3, 40; ATP, 1: KCl, 5; $MgCl_2$, 5; 1 μmole each of 19 unlabeled amino acids; phosphoenolpyruvate, 5; 0.1 to 0.15 mg of enzyme; 0.5 to 1 mg tRNA; 10 μg pyruvate kinase; 1 μc of lysine-C^{14}. For larger batches the reaction mixture was scaled up 10-fold. The mixture was incubated at 37 C for 10 min with gentle shaking. The reaction was stopped by adding an equal volume of water saturated phenol to the mixture in an icebath and extracted by the method of Gierer and Schramm (1956). The final ethanol precipitate was dissolved in 0.05 M citrate-phosphate buffer, pH 5.5, and kept at -20 C. The method of Carbon et al. (1965) was used for oxidation and reactivation of the lysyl-C^{14}-tRNA.

Preparation of MAK column: The method is essentially that of Sueoka and Yamane (1962) and the assay method was described in detail previously (Kaneko and Doi, 1966).

Assay of lysyl-C^{14}-tRNA binding to poly A-ribosome complex: The method of Nirenberg and Leder (1964) was used for the binding experiments. The only exception was the omission of mercaptoethanol during preparation of the ribosomes.

Materials: Reagents were obtained from the following sources: Schwarz Bioresearch, Inc. - L-lysine-C^{14} (240 mc/mmole); Calbiochem - 2-phosphoenolpyruvate, pyruvate kinase (rabbit muscle), ATP, CTP; Sigma Chemical Co. - poly A.

RESULTS AND DISCUSSION

The presence of sulfur-containing pyrimidines in the tRNA of B. subtilis has been described previously (Goehler et al., 1966). As Carbon

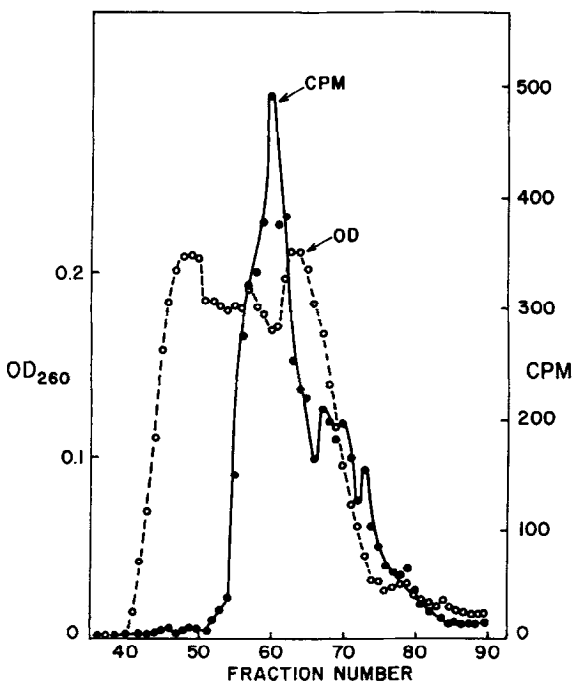

Figure 1. The elution profile of untreated lysyl-C^{14}-tRNA from a MAK column.

et al. (1965) showed, initially, mild oxidation of tRNA containing thiopyrimidines caused a loss of amino acid accepting ability. This loss can be reversed by thiosulfate treatment of the oxidized tRNA.

This mild oxidation treatment has been extended in the present work to tRNA already charged with an amino acid. In Figure 1 is illustrated the usual elution profile of lysyl-C^{14}-tRNA from a MAK column. In Figure 2, the results of mild oxidation of lysyl-C^{14}-tRNA upon its elution pattern from a MAK column is shown. The oxidized lysyl-C^{14}-tRNA eluted much earlier from the column. If mild oxidation treatment is followed by thiosulfate reduction, the profile illustrated in Figure 1 is obtained once again.

To test the binding function of the treated and untreated lysyl-

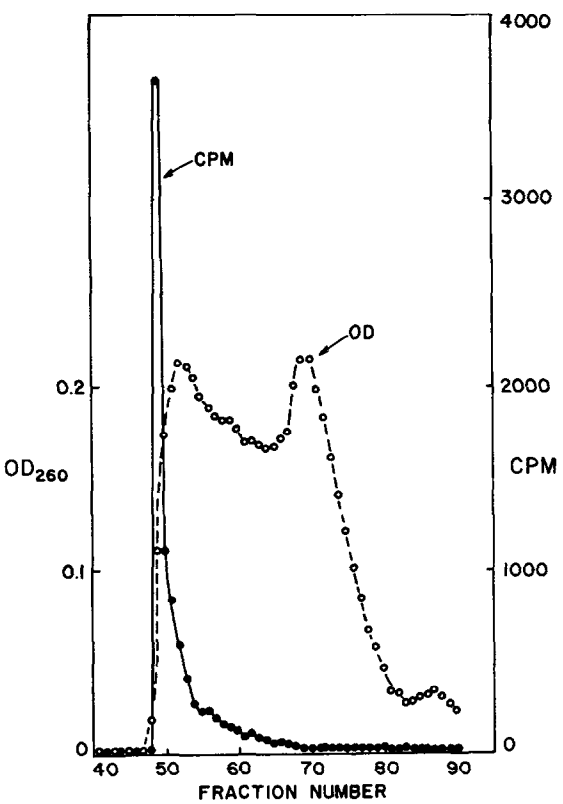

Figure 2. The elution profile of oxidized lysyl-C^{14}-tRNA from a MAK column. The oxidized lysyl-C^{14}-tRNA eluted considerably earlier from the column than the untreated lysyl-C^{14}-tRNA (see Fig. 1).

tRNAs, the method described by Nirenberg and Leder (1964) was used to test whether there was any difference between the affinity of messenger RNA-ribosome complex with the two types of aminoacyl-tRNA. Table 1 shows that the oxidized lysyl-tRNA has considerably less affinity to the poly A-ribosome complex than the untreated lysyl-tRNA. Reactivation of the oxidized lysyl-tRNA with thiosulfate treatment restores 99% of the binding efficiency.

TABLE 1

Type of lysyl-C^{14} tRNA	µµmoles of lysyl-C^{14} bound to poly A-ribosome complex	% of untreated
Untreated	7.5	100
Oxidized	2.6	35
Oxidized, reduced	7.4	99

The reaction mixtures contained in 0.05 ml: 0.1 M Tris-acetate, pH 7.2; 0.05 M KCl; 11.7 A^{260} units of ribosomes in 0.10 M Tris-acetate, pH 7.2; 0.02 M magnesium acetate; and 0.05 M KCl; and either untreated lysyl-C^{14}-tRNA (A^{260} units, 1.5; 2,050 cpm), oxidized-lysyl-C^{14}-tRNA (A^{260} units, 1.9; 2,290 cpm), or oxidized-reduced lysyl-C^{14}-tRNA (A^{260} units, 1.2; 1,840 cpm). No Mg^{++} was added besides that present in the ribosome preparation. The values above are net values with the poly A and ribosome controls substracted before calculation of the µµmoles bound to the ribosome-poly A complex.

The following points are illustrated by these results:

1. The oxidation of lysyl-tRNA affects its elution pattern from a MAK column.

2. The binding capacity of the oxidized lysyl-tRNA to the poly A-ribosome complex is reduced; this effect can be reversed almost completely by thiosulfate reduction.

The results suggest that the conformation of tRNA is important in the messenger RNA triplet recognition process. Furthermore, these results suggest a mechanism which can possibly explain the control of protein synthesis (repression) at the translation level as suggested by the previous results of Schlesinger and Magasanik (1964) and Eidlic

and Neidhardt (1965).

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