Terminal oxidation-reduction of N-acetyl-phenylalanyl-tRNA blocks initiation complex formation with *Escherichia coli* 30S ribosomal subunits

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ABSTRACT

Terminally oxidized-reduced tRNA^{Phe} of yeast, exclusively acylated at the 2'-hydroxyl of the 3'-terminal ribose of the tRNA, is a useful model for investigating the stereospecificity of AA-tRNA in protein synthesis. In this work, the ability of N-acetyl-Phe-tRNA^{ox-red} to form an initiation complex with *Escherichia coli* 30S ribosomal subunits was investigated. Thirty per cent of added control N-acetyl-Phe-tRNA was bound in a reaction dependent on initiation factors, GTP, and poly U, but no binding of the oxidized-reduced analog could be detected. These results imply that initiation complex formation may be specific for the 3'-ester of initiator tRNA.

INTRODUCTION

In previous work we have shown that terminally-modified tRNA (Fig. 1) has lost the ability to function in a number of the intermediate reactions of protein synthesis. EFTu-GTP recognition and enzymatic binding to the A site on the ribosome were blocked by this modification (1) as well as the ability to function at either the P or A site in the peptidyl transferase reaction (2). However, non-enzymatic binding to ribosomes at the P site (i.e., in the presence of tetracycline) was not affected when carried out at 15-20 mM Mg⁺⁺ (1) and the same ribosomal proteins, L2 and L27, were labelled when bromoacetyl phenylalanyl-tRNA^{ox-red} was bound to ribosomes as when control tRNA was used (3). The latter observation suggests that oxidized-reduced tRNA binds to the P site with a similar orientation as control tRNA.

In view of these facts, we have examined the initiation factor dependent binding of initiator tRNA to 30S subunits in order to see if this reaction would

be unaffected by terminal oxidation-reduction, like P site binding, or would be blocked, as in EFTu-GTP dependent binding to the A site. We have chosen to study N-acetyl-phenylalanyl-tRNA, a mimic of formylmethionyl-tRNA in the initiation of in vitro protein synthesis (4), because of our previous extensive studies on this particular substrate. This report describes the results of our experiments.

MATERIALS AND METHODS

N-acetyl-Phe-tRNA (both control and oxidized-reduced, Fig. 1) were prepared as described previously (2). Washed 30S ribosomal subunits of E. coli were prepared according to Miskin et al (5), and crude initiation factors were prepared as described by Weissbach et al (6). Formation of an initiation complex was assayed in a 50 μ l reaction mixture containing 50 mM Tris-acetate pH 7.4, 50 mM KOAc, 4 mM $MgCl_2$, 1 mM GTP, 3.6 A_{260} units of 30S subunits, 200 μ g of crude initiation factors, 10 μ g of poly U and the indicated amount of N-acetyl-Phe-tRNA. After incubation at 22° for the indicated time, the mixture was diluted with 1 ml of cold wash buffer (50 mM Tris-acetate, pH 7.4, 160 mM NH_4Cl , and 12 mM $MgCl_2$), and passed through a Millipore filter. After 3 washes with 2 ml of cold buffer, the filter was dissolved in 10 ml of Bray's (7) solution and counted.

RESULTS AND DISCUSSION

In E. coli, the first ribosome-bound complex in the initiation of protein synthesis consists of a formylmethionyl-tRNA-mRNA-30S ribosomal subunit complex formed in a sequential series of reactions requiring GTP and the presence of initiation factors. N-acetyl-Phe-tRNA can substitute for the true initiator tRNA (4) and has been used here because of our previous extensive studies on this particular tRNA in other partial reactions of protein synthesis. Table I demonstrates that N-acetyl-Phe-tRNA forms an initiation complex quite efficiently (30% bound) at 4 mM Mg^{++} and requires the presence of initiation factors, GTP, and poly U. The oxidized-reduced form of N-acetyl-Phe-tRNA

N-Acetyl-Phe-tRNA^{OX-RED}N-Acetyl-Phe-tRNA^{CONTROL}

Fig. 1. Structure of the terminus of oxidized-reduced and control N-acetyl-Phe-tRNAs. The position of acylation of tRNA^{ox-red} has recently been determined (2,9); that of the control is probably a mixture of the 2' and 3' isomers.

TABLE I

ENZYMATIC BINDING OF N-ACETYL-PHE-tRNA^{ox-red} AND
N-ACETYL-PHE-tRNA^{control} TO 30S SUBUNITS

System	N-Acetyl-Phe-tRNA Bound	
	Control	Oxidized-Reduced
	pmoles	pmoles
Complete	6.32	0.05
-GTP	1.32	0.01
-initiation factors	1.28	0.02
-poly U	0.26	0.04

Binding was assayed as described in Materials and Methods after incubation at 22° for 8 min with 20 pmoles of N-acetyl-Phe-tRNA.

completely failed to form a stable complex under these conditions. This effect is more clearly shown in Fig. 2 which illustrates both the kinetics and tRNA dependency of the binding reaction. The modified tRNA was completely inactive in this reaction, while the control functioned very well. It should be emphasized that the control tRNA was put through the same regimen of oxidation and reduction, except that the terminus was protected by attachment of phenylalanine prior to oxidation. The phenylalanine was removed before the reduction step was carried out, and subsequently replaced.

At least two alternative explanations exist for this loss of activity upon opening of the ribose ring of the terminal adenosine of tRNA. On the one hand, the conformation around the amino acid may be sufficiently altered so as to block initiation factor or ribosomal site recognition. Since initiation factors can distinguish between formylated and unformylated Met-tRNA (8), interaction between the amino acid end of tRNA and initiation factors is likely, and could be dependent on an intact ribofuranose ring. The initiator tRNA binding site itself probably does not discriminate against oxidized-reduced tRNA^{Met} since both Phe-tRNA^{ox-red} (1) and N-Acetyl-Phe-tRNA^{ox-red} (3) bind to the P site, generally considered equivalent to the initiation site, about as well as controls. In addition, the same ribosomal proteins were labelled when bromo-acetyl-phenylalanyl-tRNA (control or oxidized-reduced) was used (3). As an alternative explanation, failure to recognize N-acetyl-Phe-tRNA^{ox-red} may be due to a specificity of initiation factors for the 3'-isomer since it is now known that enzymatic acylation of tRNA^{ox-red} exclusively acylated the 2' position (2,9).

The stereospecificity of intermediate reactions in protein synthesis with respect to the position of the amino acid on tRNA has become of renewed interest with the recent finding that enzymatic acylation takes place exclusively on the 2'-hydroxyl (2,9,10). The stereospecificity of the other partial

Fig. 2. Initiation factor dependent binding of N-acetyl-Phe-tRNA^{ox-red} to 30S subunits.

- A. Time course. The reaction mixture and assays was as described in Experimental using 18 and 20 pmoles of control and oxidized-reduced N-acetyl-Phe-tRNA, respectively. The amount of radioactivity retained on the filter in the absence of 30S subunits was subtracted from each value. ●, control; ○, oxidized-reduced.
- B. N-acetyl-Phe-tRNA concentration dependence. Components of the binding mixture were as in A except that the amount of tRNA was varied as indicated. Incubation was at 22° for 8 min. ○, control; ●, oxidized-reduced.

reactions of protein synthesis are not known with certainty, although there is some indication that EFTu-dependent binding to the ribosomal A site may either be 2'-specific or non-stereoselective (11,12). The stereospecificity of peptidyl transferase at the P site is likewise not known, although it has been shown by the use of different model substrates that the A site is 3'-specific (2,13-15). Our data would indicate that initiation factor recognition may also be specific for the 3'-ester of AA-tRNA, although we cannot at this time rule out the possibility that the lack of activity is caused by structural alterations correlated with the opening of the ribose ring.

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