Detection of a 16S rRNA · initiator-tRNA complex by a new selective labelling method

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Abstract

Cupric-ion induced hydrolysis of [35S]Met-tRNA but not of N-formyl-Met-tRNA\textsubscript{Met} permitted the specific terminal labelling of initiator tRNA. Initiator tRNA, labeled in this way, was suitable for sequence analysis without the need for further purification. By probing labeled initiator tRNA with specific RNases, changes in this molecule during its interaction with the 30S particle or with 16S rRNA were investigated. Initiation complexes were resistant to the action of single-strand, base-specific nucleases Bc and Phy M and, except for one base of the anticodon stem, were also resistant to digestion by the double-strand-specific V\textsubscript{I} nuclease of Naja venom. In contrast, T\textsubscript{I} RNase digestion of the initiator tRNA in the presence of 16S rRNA enhanced cleavage of bases in the T stem of the molecule.

Introduction

Although it has long been recognized that the interplay of RNA and protein on ribosomes is responsible for the highly efficient and ordered synthesis of proteins, the specific reactions that govern each facet of this process have been difficult to define. A simpler approach to study the mechanism of translation is to predict that RNA · RNA interactions are important and to define the interplay of tRNA, mRNA and rRNA during protein synthesis (22). Interactions of 16S rRNA with mRNA (18, 20) tRNA (15) or 23S rRNA (1) have been described but the dynamics of the initiation reaction have remained essentially unexplored.

In this communication we report a method for monitoring structural changes of fMet-tRNA. Using this method, the interactions of fMet-tRNA with the 30S particle and with rRNA were studied with a number of single- and double-strand-specific nucleases. We find that the T stem/loop of fMet-tRNA interacts with a region(s) of 16S rRNA in the presence or absence of the 30S proteins or initiation factors.

Materials and methods

N-hydroxy succinimide and dicyclohexylcarbodiimide were purchased from Sigma. [35S]Met (600 Ci/mmol) from Amersham, anhydrous formic acid and dioxane from Triton Fluka. Acrylamide, bis-acrylamide and TEMED were from Biorad. Ribonucleases T\textsubscript{I} (A. oryzae) (G specific); Bc (B. cereus) (U, C specific), Phy M (Physarum polycephalum) (U, A specific), CL\textsubscript{3} (Gallus gallus) (C specific) were purchased from BRL, U\textsubscript{2} (Ustilago sphaerogena) (A specific) from P-L Biochemicals and V\textsubscript{I} (Naja naja oxiana) (double-strand specific) from Sigma. Crude tRNA and pure tRNA\textsubscript{Met} from E. coli B were purchased from Schwartz/Mann and Sigma, respectively. AUG and MS2 RNA were bought from Miles.

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Acylation and formylation of f[35S]Met-tRNA^Met

Unpurified *E. coli* tRNA was aminoacylated with [35S]Met and formylated enzymatically (7), while pure tRNA^Met was aminoacylated (8) with [35S]Met and formylated chemically (19) with the following modifications. N-formyl succinimide was prepared by dissolving 5.75 g of N-hydroxy succinimide and 1.9 ml of dry formic acid in 100 ml of dry dioxane. Dicyclohexylcarbodiimide (10.3 g) was dissolved in 50 ml dioxane. These two solutions were combined and stirred for 30 min. The resulting precipitate was filtered and washed with 5 ml dioxane. Then the filtrates were rerecrystallized from 50 ml boiling ethanol. The product was dried and stored desiccated at -20 °C. [35S]Met-tRNA^Met was dissolved in 0.2 ml H2O and added to 0.8 ml TEAE buffer (1.4% triethylamine, pH 8.0/5 mM MgCl2). The mixture was immediately transferred with constant stirring to 1 ml N-formyl succinimide-dioxane solution (0.125 g/ml dry dioxane) and the pH was adjusted to 8.0 with 2.0 N KOH. The mixture was left for 20 min at 24 °C, made 0.2 M with KAc, pH 5.0, and the pure f[35S]Met tRNA^Met was precipitated with 2 volumes of EtOH at -20 °C. After centrifugation at 27 000 × g for 10 min, the pellet was redissolved in a minimum volume of water, dialyzed against 10 mM KAc pH 5.0/0.5 mM EDTA and then against H2O.

Cupric-ion treatment of aminoacyl-tRNA

Aminoacyl-tRNAs are preferentially hydrolyzed by cupric-ion treatment while N-acyl-aminoacyl-tRNAs are not hydrolyzed (11). Unpurified f[35S]-Met-tRNA containing [35S]Met-tRNA was incubated with fresh 10 mM CuSO4 in 0.25 M KAc, pH 5.0 at 87 °C for 3.5 min. After adding 0.2 volumes 5 M NaCl, EDTA was added to a final concentration of 11 mM. The RNA was ethanol precipitated, washed with 0.5 ml 75% EtOH/1 mM EDTA, pH 7.4/1 mM MgCl2 at -20 °C and washed again with 75% EtOH/2 mM KAc, pH 4.5/1 mM MgCl2 at -20 °C. The suspension was centrifuged, dried with nitrogen and suspended in a minimum volume of H2O. This procedure decaysates approximately 95% of the aminoacyl-tRNA as measured by TCA precipitable counts of [14C]phe-tRNA while leaving fMet-tRNA intact. The extent of formylation was determined as described (8).


30S particles (40 mg/ml) were first isolated, activated (8) and adjusted to give a final concentration of 50 mM Tris, pH 7.4/50 mM NH4Cl, pH 7.4/4.25 mM MgCl2/15 mM DTT/1 mM GTP (Buffer A). The particles were phenol-extracted twice, extracted with ether, made 0.2 M with KAc, pH 5.0, and ethanol precipitated at -20 °C. The 16S rRNA thus obtained was dissolved in sterile H2O. Varying concentrations of 16S rRNA were added to 0.18 pmole of f[35S]Met-tRNA and 1.8 units of RNase T1 in 5 μl of 50 mM NaAc pH 5.0/1 mM EDTA and incubated at 24 °C for 20 min. The samples were loaded on a 20% polyacrylamide gel containing 7 M urea. 37 pmoles of activated 30S particles were used instead of 16S rRNA in some experiments. These were added to 0.34 pmole f[35S]Met-tRNA and 500 pmoles AUG or 40 μg MS2 in .025 ml Buffer A. Mixtures were frozen at -70 °C, lyophylized and resuspended in 10 μl 50 mm NaAc pH 5.0, 1 mM EDTA, 7 M urea and 1.8 U T1, incubated 20 min at 24 °C and loaded onto a 20% sequencing gel.

Preparation of initiation complexes

Initiation complexes were formed in Buffer A by mixing 74 pmoles of 30S particles, .065 pmoles f[35S]Met-tRNA, 500 pmoles AUG or 40 μg MS2 RNA, 1.3 μg IF-1, 20 μg IF-2, and 6.4 μg IF-3 in a final volume of 25 μl (8). After incubation at 24 °C for 15 min, 80–90% of the f[35S]Met-tRNA was bound to 30S particles as determined by the nitrocellulose filter assay (12). IF-1, 2 and 3 were needed to obtain optimum binding of f[35S]Met-tRNA to 30S particles programmed by AUG or MS2 RNA. These complexes were then exposed to varying amounts of single-strand or double-strand specific-nucleases. Reactions were stopped by addition of 75 μg/ml tRNA (E. coli B) and ethanol precipitation or lyophylization. For the case of T1, reactions were phenol extracted so that cutting would not occur while loading samples. The pellets were resuspended in 7 M urea/50 mM NaAc, pH 5.0/1 mM EDTA/20% sucrose and loaded onto 15% or 20% polyacrylamide (37.5:1 bis) gels containing 7 M urea.