Effect of Nascent Peptide Steric Bulk on Elongation Kinetics in the Ribosome Exit Tunnel

Pengse Po [1], Erin Delaney [1], Howard Gamper [2], D. Miklos Szantai-Kis [4], Lee Speight [3], LiWei Tu [1], Andrey Kosolapov [1], E. James Petersson [3], Ya-Ming Hou [2] and Carol Deutsch [1]

*1 - **Department of Physiology,** University of Pennsylvania, Philadelphia, PA 19104, USA*
*2 - **Department of Biochemistry and Molecular Biology,** Thomas Jefferson University, Philadelphia, PA 19107, USA*
*3 - **Department of Chemistry,** University of Pennsylvania, Philadelphia, PA 19104, USA*
*4 - **Department of Biochemistry and Molecular Biology,** University of Pennsylvania, Philadelphia, PA 19104, USA*

***Correspondence to Carol Deutsch:** Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104-6085, USA. cjd@mail.med.upenn.edu*
http://dx.doi.org/10.1016/j.jmb.2017.04.019
Edited by Ruben L. Gonzalez

Abstract

All proteins are synthesized by the ribosome, a macromolecular complex that accomplishes the life-sustaining tasks of faithfully decoding mRNA and catalyzing peptide bond formation at the peptidyl transferase center (PTC). The ribosome has evolved an exit tunnel to host the elongating new peptide, protect it from proteolytic digestion, and guide its emergence. It is here that the nascent chain begins to fold. This folding process depends on the rate of translation at the PTC. We report here that besides PTC events, translation kinetics depend on steric constraints on nascent peptide side chains and that confined movements of cramped side chains within and through the tunnel fine-tune elongation rates.

Introduction

The ribosome is endowed with an exit tunnel through which the newborn peptide moves during chain elongation. At one end, the peptidyl transferase center (PTC), translation of mRNA occurs and peptide bonds form. At the other end, the exit port, the elongating nascent chain emerges. In between, the nascent peptide begins to fold [1–11]. The tunnel, made of rRNA, some protein, water, and ions, plays an active role in early protein folding [12–14]. The efficiency of this biogenic process depends critically on the rate of translation, which varies as the protein is synthesized [15–20], and also depends on the length of a nascent peptide [8–11,21], slow *versus* fast translating codons [22–26], mRNA conformations [27,28], and interactions of a nascent peptide with tunnel components and chaperones [29]. As the peptide wends its way through the ribosome's heterogeneous corridor, ~100-Å long and 10~20-Å wide [30,31], it will encounter distinctive variations and "constrictions". Each amino acid side chain will thus traverse a unique energy landscape along the tunnel walls and will seek a preferred microenvironment, dependent on the physicochemical properties of both the peptide side chain and the tunnel. Any twisting/turning/compaction of the peptide will alter peptide–tunnel interactions, especially in the more confined regions of the tunnel (e.g., the constriction, which is estimated to have a diameter of ~10 Å). In the case of so-called arrest peptides, peptide–tunnel interactions relay a signal to the PTC to produce conformational changes that inhibit translation [32–44]. It is likely that similar sensing and relay mechanisms are used for all peptides, not just for evolutionarily programmed arrest sequences. Besides backbone rearrangements (e.g., kinks and helical turns) [2–4,8] of the nascent peptide within the tunnel, we hypothesize that the size [13], electrostatic properties [41,44,45], and orientations of side chains will influence the kinetics of elongation.

Clearly, the tunnel can accommodate large side chains, introduced either by genetic encoding or by chemical reaction. The former includes unnatural

amino acids [2,46–50]; the latter includes large cysteine (Cys) reagent molecules, for example, trialkylammonium maleimides that can enter the tunnel and react with nascent peptide Cys [13,51]. Although all of these reagents completely react with Cys engineered into the nascent peptide, the modification rates of each of these reagents decrease monotonically with their increasing molecular volumes, from 270 to 440 $Å^3$ [13]. This result supports the idea that covalent modification is kinetically restricted by steric factors within the tight confines of each tunnel location. Moreover, side-chain sterics promote both short-range and long-range rearrangements of the nascent peptide in the tunnel [13,14]. While these results suggest a steric component to the modification reactions in the tunnel, they also raise two important questions. First, does the size and nature of a peptide side chain moving within and through the tunnel alter elongation rates? If so, could this be mediated by a feedback mechanism that governs the kinetic and thermodynamic state of the peptide in the P-site, a site at the PTC that binds peptidyl-tRNAs? Second, can the extent of this modulation be accounted for by the physicochemical interactions between peptide side chains and the tunnel walls? To investigate these possibilities, we developed a two-step translation strategy to incorporate natural or unnatural test amino acids into the peptide in the first step and then further elongate the chain synchronously in a second step. Note that we will use the term "elongation" to refer to the overall process of resumed translation. This strategy allowed us to introduce side chains of systematically increasing size and to separate effects on amino acid incorporation from effects on elongation. To determine putative side-chain-dependent kinetic effects, we used both natural and unnatural amino acids introduced along the peptide at different tunnel locations. Unnatural amino acids provided the opportunity to systematically alter the size of the side chain with minimum permutation of other properties.

Our results show that at sensitive locations within the tunnel, side-chain size inversely correlates with elongation rate and that the effect can be accounted for quantitatively by van der Waals interactions between the introduced amino acid and the tunnel walls. These studies suggest that peptide–tunnel interactions can modify the energetics and kinetics of processes that underlie conformational rearrangements at the PTC during peptide bond formation.

Results

Arrest and rescue of nascent chains

Native Cys in a nascent peptide can be covalently modified by alkylammonium maleimides (X) while residing in the ribosome exit tunnel [13]. If these alkylammonium moieties were incorporated as unnatural amino acids, X-Cys, and traveled through the tunnel as a peptide is elongated, then we could exploit the systematic increase in size of alkyl groups (methyl, ethyl, butyl) to investigate the effect of nascent peptide side-chain sterics on elongation kinetics. Two prerequisites are necessary: (i) synthesis of unnatural X-Cys and their corresponding aminoacylated tRNA (X-Cys-$tRNA^{Cys}$), and (ii) a means to selectively incorporate X-Cys and measure the kinetics of subsequent chain elongation. The construct used in all experiments was an mRNA that encodes for a nascent peptide of 173 aa [9] with a single Cys codon at position 123 but lacks a stop codon. Translation was carried out in a cell-free rabbit reticulocyte lysate (RRL) system to generate a nascent peptide that remains attached to the ribosome due to the lack of a stop codon in the mRNA.

To satisfy our first prerequisite, we designed and synthesized a series of trialkylammonium-decorated ethylmaleimides, namely, *trimethyl*ammonium (TMA), *triethyl*ammonium (TEA), and *tributyl*ammonium (TBA), with a range of sizes 270–440 $Å^3$ (Fig. 1). Because maleimides inhibit protein synthesis, these probes could not be used as naked maleimides during translation. Therefore, prior to translation, we covalently linked *N*-ethylmaleimide (NEM) or NEM decorated with TMA, TEA, and TBA (X) to Cys. These modified Cys could then be attached to tRNA and used in subsequent translation reactions. To synthesize the unnatural amino acid attached to tRNA, we used an *Escherichia coli* $tRNA^{Cys}$ transcript [52] and a flexible ribozyme for tRNA acylation developed by Suga and coworkers (flexizyme, dFx) [53]. The flexizyme facilitates the aminoacylation of tRNA with amino acid substrates (modified or unmodified Cys) that have been activated by an attached 3,5-dinitrobenzyl ester (DBE; Fig. 1). DBE provides a good leaving group, which allows the tRNA aminoacylation reaction to proceed for a wide variety of unnatural amino acids [53]. The broad substrate range of dFx was optimal for us to use as a catalyst for tRNA aminoacylation. The charging efficiency of each flexizyme reaction ranged from 7 to 20% for DBE substrates of both modified and unmodified Cys (Fig. S1).

Regarding our second prerequisite to selectively incorporate X-Cys and subsequently determine elongation kinetics, we depleted the RRL of available Cys-$tRNA^{Cys}$ by inhibiting endogenous RRL cysteinyl-tRNA synthetase (CysRS). To inhibit CysRS, we made a nonhydrolyzable aminosulfonamide (Cys-AMS; Fig. 2a), according to the chemical protocols used by Tan and coworkers [54,55] (see Materials and Methods). The efficacy and specificity of this Cys-AMS inhibitor were evaluated using purified tRNA synthetases (RS) from *E. coli* (*Ec*) or human (*Hs*), specific for Cys (*Ec*CysRS), lysine (*Hs*LysRS), glycine (*Hs*GlyRS), and alanine (*Hs*AlaRS) for the inhibitor