Ribosomes

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Peptide Bond Formation on the Ribosome: The Role of the 2′-OH Group on the Terminal Adenosine of Peptidyl-tRNA and of the Length of Nascent Peptide Chain

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Ribosome is responsible for precise translation of genetic information into proteins, which are essential in all living organisms. The peptidyl transferase center (PTC) on the large ribosomal subunit catalyzes the synthesis of peptide bonds. Reaction of the α-amino group of aminoacyl-tRNA on the A-site with the carbonyl group of peptidyl-tRNA on the P-site results in transfer of the nascent peptide from peptidyl-tRNA to the aminoacyl-tRNA and elongation of the peptide by one amino acid residue. Structural research on ribosomes together with biochemical studies led to attempts to predict the catalytic mechanism of peptide bond formation.[1–4] One of the most recent hypotheses by Weinger et al. is the substrate-assisted catalysis in which the 2′-OH group on the 3′-terminal adenosine of peptidyl-tRNA participates in the proton shuttle during the peptide-bond formation (Figure 1). An almost 10^6-

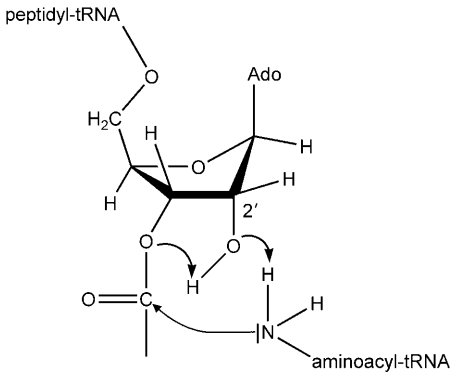

Figure 1. Suggested mechanism for the participation of the 2′-OH group of the 3′-terminal adenosine residue (Ado = adenine residue) of peptidyl-tRNA in peptidyl transfer.

fold reduction in the rate of peptide bond formation is found for puromycin bound to an A-site if this 2′-OH group is removed or replaced by fluorine in the P-site-bound peptidyl-tRNA.[3] In support of this mechanism, the structure analysis of the 70S ribosome from *Thermus thermophilus* with a complete PTC including an A-site-bound aminoacyl-tRNA, showed that the 2′-OH of A76 of the peptidyl-tRNA is within hydrogen-bonding distance of the α-amino group of aminoacyl-tRNA on the A-site.[5] Short synthetic oligonucleotides were usually used as mRNA in in vitro biochemical analyses and X-ray structure determination.[3,5] In addition, in many cases, tRNA fragments or substrate analogues were used as models for aminoacyl- and peptidyl-tRNA. In contrast to these reports, when complete peptidyl-tRNA-2′dA and full-length mRNA were applied in the in vitro translation assays, the rate of peptide bond formation during the translation of an internal codon at position 155 of a 310 amino acid long polypeptide chain of esterase 2 from *Alicyclobacillus acidocaldarius* (Est2) was not significantly inhibited by removal of the 2′-OH group from the peptidyl-tRNA on the P-site.[6] The main difference between the translation of an internal codon of a full-length mRNA and a model experiment with short synthetic mRNA is in the occupation of peptide exit channel of the 50S subunit. It is documented that the length of the nascent peptide chain changes the accessibility of the PTC to antibiotics and affects the outcome of cross-linking experiments in the PTC.[7]

We assumed that the lack of the 2′-OH group on A76 of peptidyl-tRNA may influence the conformation of the peptide donor if the nascent peptide chain is still short and not anchored in the peptide exit channel. This arrangement could explain the different results reported for model experiments with purified components of translation machinery and those from complete translation systems.[3,6]

To demonstrate that the effect caused by removal of 2′-OH on A76 of peptidyl-tRNA is dependent on the length of the nascent polypeptide chain, we introduced a RF1-dependent, nonsense UAG codon at the 5′-terminus of mRNA of Est2 in the region coding for N-terminal amino acids at positions 2, 4, 6, 8, 10, 14, 26, and 155. Suppression of these codons by suppressor $tRNA^{Ser(CUA)}$-2′dA and suppressor $tRNA^{Ser(CUA)}$-A in the in vitro translation of complete Est2 mRNA was measured by esterase activity. If the substrate-assisted catalysis mechanism involving the 2′-OH group of peptidyl-tRNA during peptide transfer is correct, the replacement of $tRNA^{Ser(CUA)}$-A with $tRNA^{Ser(CUA)}$-2′dA should have a similar effect at all positions and the in vitro synthesis of Est2 with a nonsense codons should be prevented independently of the position of UAG codons in the mRNA.

$tRNA^{Ser(CUA)}$-2′dA was prepared as previously described[6,8] and double treated with excess periodate to oxidize any remaining tRNA that terminated with ribose. $tRNA^{Ser(CUA)}$-2′dA was aminoacylated up to 270 pmole/A_{260} by seryl-tRNA synthetase from *E. coli*.

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Est2 was synthesized in a coupled in vitro *E. coli* transcription/translation system in the presence either of $tRNA^{Ser(CUA)}$-A or $tRNA^{Ser(CUA)}$-2′dA and mRNA with UAG stop codons in different positions. When the ribosomal A-site encounters a stop codon, premature termination, frame shifting, or suppression takes place.[9,10] To increase the efficiency of suppression in the presence of suppressor $tRNA^{Ser(CUA)}$, release factor 1 (RF1) was removed from the in vitro transcription/translation system by adding an antibody against RF1. This treatment results in almost complete suppression of premature termination and efficient incorporation of serine into the UAG coded positions.[11] However, in the absence of suppressor $tRNA^{Ser(CUA)}$, beside stalling and frame shifting, suppression with an endogenous suppressor aminoacyl-tRNA, such as Tyr-$tRNA^{Tyr}$ (codon UAY) may occur.[12] Since the replacement of amino acids in the N-terminal region of Est2 is not detrimental for the esterase activity, a small amount of active enzyme can be detected also under the conditions without suppressor tRNA (Figures 2 and 3). However, when serine residue 155, which is essential in the catalytic triad of Est2,[13] was replaced by the UAG codon, no esterase activity was detected in the absence of $tRNA^{Ser(CUA)}$. Active esterase can only be synthesized through the suppression of UAG-155 by Ser-$tRNA^{Ser(CUA)}$.[6] Kinetic measurements of the in vitro translation of esterase with the stop codon at the 2nd position or the 155th position of the amino acid sequence of Est2 showed that even after 30 min of incubation, the peptide synthesis is still in the linear phase (Figure 2).

In the presence of $tRNA^{Ser(CUA)}$-A the suppression of the UAG nonsense codon occurred efficiently in all the tested positions and the active esterase was synthesized to a great extent. Compared with experiments in the absence of suppressor tRNA, a 6–26-fold increase of esterase activity was detected in the presence of $tRNA^{Ser(CUA)}$-A when the UAG codons were located at positions coding for the N-terminal amino acids of Est2. This effect varied with the position of the UAG codon in the 5′-region of Est2 mRNA (Figure 3).

If $tRNA^{Ser(CUA)}$-2′dA was used to replace $tRNA^{Ser(CUA)}$-A in the in vitro translation, the suppression of the UAG codon also took place. However, the suppression of UAG stop codons at the first 10 amino acids downstream of the AUG

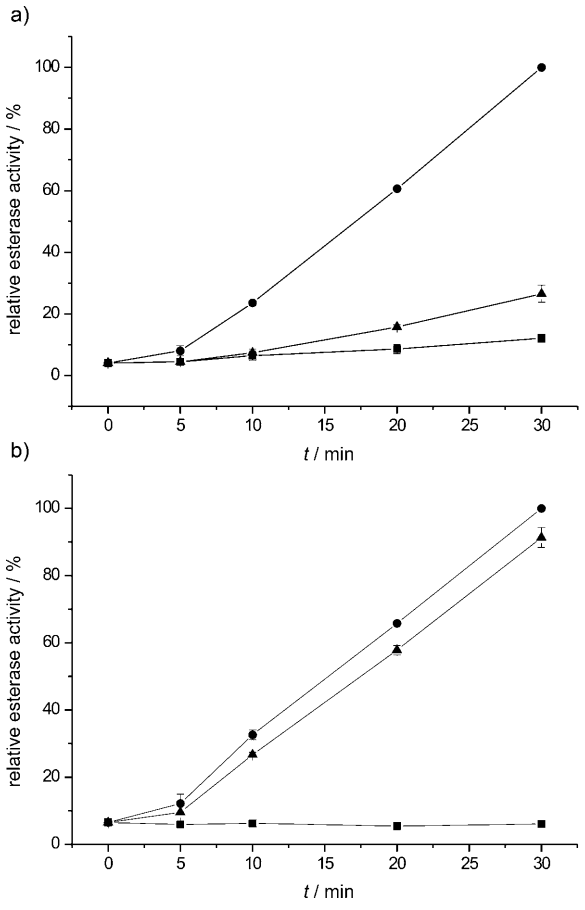

Figure 2. Kinetics of the in vitro synthesis of active Est2. a) In vitro synthesis of Est2A2X. b) In vitro synthesis of Est2S155X. The activity of Est2, withdrawn at the indicated time intervals, was monitored photometrically.[9] ■ without $tRNA^{Ser(CUA)}$, ● with $tRNA^{Ser(CUA)}$-A, ▲ with $tRNA^{Ser(CUA)}$-2′dA. Error bars show standard deviations ($n=3$).

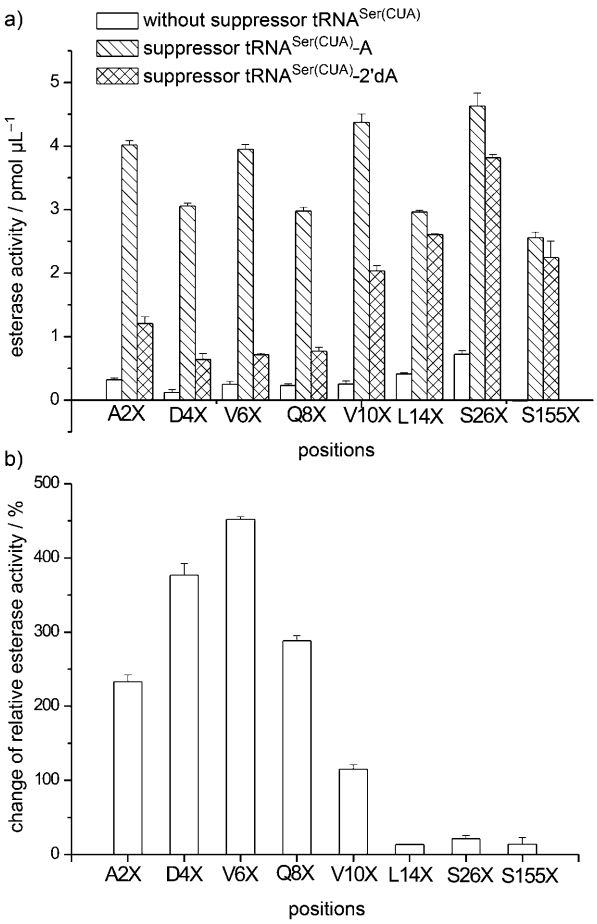

Figure 3. Suppression of the UAG stop codons in positions 2, 4, 6, 8, 10, 14, 26, and 155 of Est2 mRNA by $tRNA^{Ser(CUA)}$-A or $tRNA^{Ser(CUA)}$-2′dA. Letters indicate the amino acid originally present in Est2, which were replaced by serine from seryl-$tRNA^{Ser(CUA)}$. a) Suppression activity by the presence of $tRNA^{Ser(CUA)}$-A compared to $tRNA^{Ser(CUA)}$−2′dA with stop codons at different positions; b) enhancement of suppression activity in the presence of $tRNA^{Ser(CUA)}$-A as compared to $tRNA^{Ser(CUA)}$ −2′dA. Error bars show standard deviations ($n=3$).

start codon of Est2 mRNA by $tRNA^{Ser(CUA)}$-2′dA was less efficient than by $tRNA^{Ser(CUA)}$-A. In these positions, esterase activity was 50–80 % lower with $tRNA^{Ser(CUA)}$-2′dA than with $tRNA^{Ser(CUA)}$-A. In other words, the presence of the 2′-OH group on A76 of $tRNA^{Ser(CUA)}$ facilitates the peptide bond formation and incorporation of the first 10 amino acids of Est2 (Figure 3). There is little difference in suppression efficiency between $tRNA^{Ser(CUA)}$-A and $tRNA^{Ser(CUA)}$-2′dA when UAG stop codons are coding for position 14, 26, or 155 of the Est2 sequence. In such cases, only an approximately 10 % increase in the yield of esterase was detected, a result which is also in agreement with our previous investigations.[6]

The results confirm our earlier finding that the 2′-OH group on A76 of peptidyl-tRNA is not essential for the peptide bond formation if complete tRNA and full-length mRNA are used. In the experiments presented herein, we show that after the initiation phase of translation, when the nascent polypeptide chain is longer than 10 amino acid residues, the suppression of the UAG nonsense codon by $tRNA^{Ser(CUA)}$ was not affected by the removal of the 2′-OH group from A76. However, when UAG stop codon is coding for positions 2 to 10 of the Est2 sequence, the 2′-OH group on A76 of $tRNA^{Ser(CUA)}$ enhanced the peptide synthesis 1–4.5-fold.

Weinger et al. demonstrated that the formation of fMet-Lys-puromycin is about 10^6-fold faster when fMet-Lys-$tRNA^{Lys}$-A is present in the ribosomal P-site than when fMet-Lys-tRNA-2′dA is in the P-site. The substrate-assisted catalysis mechanism, in which the 2′-OH group of the terminal adenosine residue of the peptidyl-tRNA located in the P-site participates in a proton shuttle to accept a proton from the incoming α-amino group and deliver it to the outgoing deacylated tRNA, was derived from this experiment. Our results do not support this mechanism. Even if the UAG stop codon is located at the 5′-end of mRNA, close to initiation codon, only a 1–4.5-fold increase of esterase activity was observed with $tRNA^{Ser(CUA)}$-A as compared with $tRNA^{Ser(CUA)}$-2′dA (Figure 3). The difference in suppression activity by $tRNA^{Ser(CUA)}$-2′dA when the UAG is located in positions near to the 5′ end of mRNA, coding for N-terminal amino acids, and in the middle of mRNA, may be due to the fact that the short nascent peptide, less than 10 amino acid residues in length cannot yet reach the peptide exit tunnel. Erythromycin, bound to the entrance of the nascent-peptide exit tunnel, allows a synthesis of peptides with lengths between six and eight amino acids. Further peptide elongation is inhibited, and peptidyl-tRNA dissociates prematurely from the ribosome in a drop-off manner.[7]

The ribose of A76 of peptidyl-tRNA may be more flexible in the case when the short nascent peptide is still not located in the peptide exit tunnel.[14] In such cases the presence of 2′-OH group on A76 of the peptidyl-tRNA may have a stronger supportive effect on peptide bond formation as is the case when the peptidyl-tRNA is additionally anchored in the PTC by interaction with peptide exit tunnel. When the nascent peptide reaches the peptide exit tunnel, the structures of substrates in PTC are sufficiently defined. This situation may indicate that ribosome achieves peptide bond formation not by involvement of functional groups in chemical catalysis but by providing the frame for precise positioning of peptidyl-tRNA and aminoacyl-tRNA.[15]

The structural research on ribosome complexes in which the nascent peptide is located in the exit tunnel and with longer mRNA may give us more information about mechanism of peptide bond formation.

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