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A theoretical model investigation of peptide bond formation involving two water molecules in ribosome supports the two-step and eight membered ring mechanism

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ABSTRACT

The ribosome is the macromolecular machine that catalyzes protein synthesis. The kinetic isotope effect analysis reported by Strobel group supports the two-step mechanism. However, the destination of the proton originating from the nucleophilic amine is uncertain. A computational simulation of different mechanisms including water molecules is carried out using the same reaction model and theoretical level. Formation the tetrahedral intermediate with proton transfer from nucleophilic nitrogen, is the rate-limiting step when two water molecules participate in peptide bond formation. The first water molecule forming hydrogen bonds with O9′ and H15′ in the A site can decrease the reaction barriers. Combined with results of the solvent isotope effects analysis, we conclude that the three-proton transfer mechanism in which water molecule mediate the proton shuttle between amino and carbon oxygen in rate-limiting step is the favorable mechanism. Our results will shield light on a better understand the reaction mechanism of ribosome.

1. Introduction

Ribosomes, universal cellular riboprotein assemblies, are the cell-machines, which translate the genetic code into proteins. Peptide bond formation reaction takes place within the peptidyl transferase center (PTC) of the 50S subunit [1–4]. As the peptide bond forms, a peptidyl tRNA which is elongated by one amino acid in the A site and a deacylated tRNA in the P site are produced [2,3,5]. The nucleophile α-amino group of the aminoacyl tRNA in the A site attacks the carbonyl ester of the peptidyl tRNA in the P site. Generally, the ribosome accelerates the rate of peptide bond formation by 10^6 to 10^7-fold [6]. This rate is probably mainly achieved through substrate proximity and alignment effects from the ribosome [6–9]. At the same time, the ribosome also has a significant chemical catalytic role by coordinating a proton transfer network. However, this chemical catalytic mechanism of peptide bond formation still remains controversial.

In recent years, kinetic [6,8,10], biochemical [11–15] and computational studies [9,16–21] have provided substantial information about the chemical basis for the peptide bond formation. Three functional groups, including the group 2′-OH of A76 in peptidyl-tRNA, the group 2′-OH of A2451 and two highly ordered water molecules, are identified to be essential for the peptide bond formation. Firstly, the group 2′-OH of A76 is proposed to mediate the "proton shuttle" from the nucleophile α-amino group of the aminoacyl tRNA to the ester O3′ leaving group [11,13,22–24]. Weinger et al. have provided an evidence that when peptidyl transferase reactions are carried out with tRNA fragments or puromycin, a very strong (10^6-fold) inhibitory effect is observed in the absence of the 2′-OH group on the peptidyl substrate [13]. However, the function of the 2′OH of A76 is questionable [14,15,25]. Zaher et al. have found that the catalysis is at least 100-fold slower with the dA76-substituted peptidyl-tRNA substrate and that the peptidyl transferase center undergoes a slow inactivation when the peptidyl-tRNA lacks the 2′-OH group of A76 [14]. However, these results cannot rule out the role of the 2′-OH A76 in peptidyl transferase reaction [26]. Secondly, the function of the group 2′-OH of A2451 is identified as orienting the ribose of A76 in the proton shuttle process by hydrogen bond to 2′-OH of A76 directly [12,27–32]. This interaction can keep a C2′-endo conformation in A76 ribose to support amide synthesis through the proton shuttle mechanism. Simultaneously, the direct interaction prevents the intra-molecular proton transfection of the peptide from the 3′- to

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2′-oxygen. Thirdly, two ordered water molecules are observed in the crystal structure with transition state analogs [1]. One water molecule is coordinated via hydrogen bonds to the developing oxyanion and two highly conserved residues (A2602 and U2584), and the second water molecule is observed in close contact with A76 and A2451. The two water molecules should be introduced in the peptide bond formation, although the function of two ordered water molecules in the ribosome remains obscure [19].

Based on experimental and theoretical results, two types of mechanisms have been proposed: the concerted mechanism and the two-step mechanism [9,16,19,21,33,34]. The concerted mechanism including six-membered, eight-membered and four-membered ring mechanisms has been theoretically evaluated. As shown in Fig. 1(a), the six-membered ring mechanism has been extensively discussed in many reports [9,16]. The 2′-OH of A76 donates its proton to the leaving group ester atom O3′, whereas the α-amino group donates one proton to the 2′-OH of A76. Thereafter, the eight-membered ring mechanism is proposed (Fig. 1(b)) [20]. This mechanism includes a double proton shuttle, in which one water molecule actively participates in the concerted six-membered ring mechanism. The function of the second water molecule is suggested to stabilize the developing negative charge of the oxyanion in the transition state. Eight-membered concerted mechanism gives an activation enthalpy in excellent agreement with experiments [6,8]. The QM/MM free energy simulations also support the eight-membered ring concerted reaction mechanism [21].

In the four-membered ring mechanism (Fig. 1(c)) [18], the α-amino group directly donates one proton to the ester O3′ leaving group. The 2′OH of A76 forms a hydrogen bond with the carboxyl oxygen of A site. If two water molecules are involved in the four-membered ring mechanism, a six-membered ring mechanism will be favorable (Fig. 1(d)) [19]. The 2′-OH of A2451 is also involved in the mechanism. One of water molecule actively participates in the proton shuttle concerted mechanism. The second water molecule also stabilizes the oxyanion in transition states. In both mechanisms, the 2′-OH of A76 does not participate in proton transfer, just serving as an anchor, which holds the reactant in place at the transition state. This type of mechanisms is in consistence with the experimental results that the contribution of the 2′OH of A76 to the rate of peptide bond formation is small (100-fold) [14].

The two-step mechanism is originally proposed by our group (Fig. 1(e1) and (e2)) [34]. In the first step (Fig. 1(e1)), the 2′-OH of A76 group receives one proton from the α-amino group. Simultaneously, it transfers one proton to the carbonyl oxygen. Protonation of the carbonyl oxygen neutralizes the negative charge of the oxyanion, resulting in a neutral intermediate. In the second step, the intermediate is resolved into the product, and the proton is transferred to the O3′ leaving group directly. The first step with the higher energy barrier determines the rate of reactions.

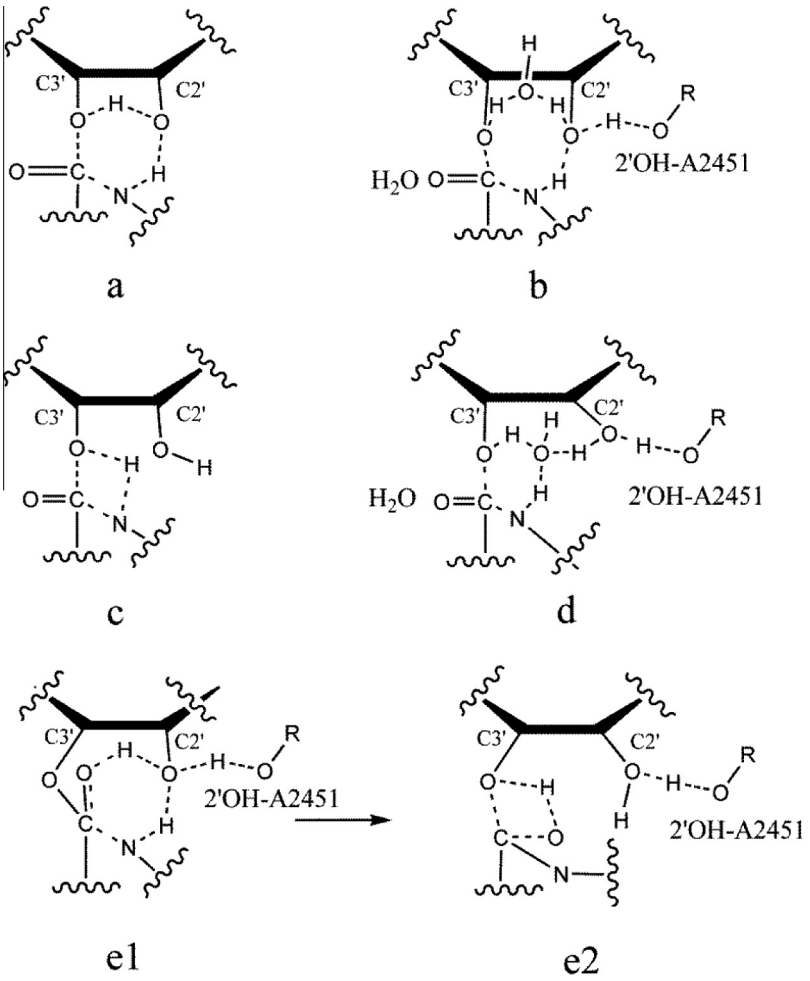

Fig. 1. Schematic representation transition state of the five studied mechanisms: six-membered (a), eight-membered (b) four-membered (c), water mediating six-membered (d), two-step mechanism (e1 and e2).