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D amino acids highlight the catalytic power of the ribosome

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Abstract

The possible mechanism(s) by which ribosomes make peptide bonds during protein synthesis have been explored for decades. Yet, there is no agreement on how its catalytic site, the peptidyl transferase center (PTC), promotes this reaction. Here we discuss results of recent investigations of translation with D amino acids that provide fresh insights into that longstanding question.

During protein synthesis on the ribosome, a new peptide bond is formed each time an amino acid linked to the 3' end of an incoming tRNA reaches the peptidyl transferase center (PTC). In this cavity at the heart of the large subunit of the ribosome, the amino acid is positioned so that its nucleophile, the amino group, can react with the ester bond connecting a nascent protein to the tRNA of the P site. Does the ribosome only bring reactive species into close proximity, or does it use special tricks to speed up the reaction? One chemical group of RNA, the 3' terminal 2'OH of the P-site tRNA, has received much attention because it is within hydrogen bonding distance of the amino group during nucleophilic attack. Early investigations suggested that it could accept a proton from the amine while giving its own proton to the 3' O of the P site tRNA from which the protein breaks away (Weinger et al. 2004, Schmeing et al. 2005a). Alternative pathways of this proton shuttle have been investigated (Polikanov et al. 2014), but no strong conclusion on the magnitude of its catalytic effect has been reached (Maracci et al. 2015, Kazemi et al. 2018). New insights into this issue unexpectedly came from recent investigations on the compatibility of the translation system with D amino acids. For a long time, it was thought that these substrates could not be properly handled by the PTC, but two practical advances led to a reevaluation of that statement. One was to work with optimal tRNAs for the delivery of the amino acid (aa) to the ribosome, that occur in the form of a [aa-tRNA•Ef-Tu•GTP] ternary complex, where aa-tRNA is an aminoacyl-tRNA and Ef-Tu is the prokaryotic elongation factor of translation. The affinity of Ef-Tu towards the amino acid is side-chain dependent (Lariviere et al. 2001), and was found to be low for D amino acids. However, this can be circumvented by working with a tRNA body with a high affinity for Ef-Tu so that a more stable ternary complex is obtained (Lariviere et al. 2001, Achenbach et al. 2015, Katoh et al. 2017). The other requirement was to be able to load D amino acids on tRNAs in the absence of specific synthetase. In a breakthrough contribution, Fujino et al. (2013) managed to achieve this with a *flexizyme*, an artificially evolved RNA that can load any amino acid onto the 3' end of a tRNA (Morimoto et al. 2011). They demonstrated that for some D amino acids including alanine, serine and cysteine, short peptides with a single D amino acid inclusion can be synthesized almost as efficiently as equivalent all-L peptides. Remarkably, time-course experiments showed that the rate of production was similar for both types of peptides for specific concentrations of aminoacyl-tRNAs (Fig. 1a). This suggested that the PTC could handle D amino acids after all. Importantly, examination of the

reactional context occurring with D enantiomers (Fig. 1b, c) revealed that the amino group cannot interact with the A76 2'OH of the P-site, not only because it is too far away (~4 Å), but also because neither of its two hydrogens is favorably oriented during nucleophilic attack (Fig. 1d). Combined with the data of Fujino et al., this suggested that the proton transfer to A76 2'OH was not critical for elongation. However, kinetic measurements of peptide bond formation with D amino acids were still required to quantitatively evaluate the effect.

These eagerly awaited results were published recently in the work by Liljeruhm et al. (2019). Working with D phenylalanine (D-Phe) and tRNA^{ala}, an aminoacyl carrier that could best compensate the low affinity of D-Phe for Ef-Tu, they established that the kinetic constant of tRNA accommodation/peptide bond formation at pH 7.5 was ~50 fold slower with D-phe-tRNA^{ala} compared to L-phe-tRNA^{ala} under an optimal concentration of Ef-Tu. Although no experimental approach has allowed discrimination between tRNA accommodation and peptide bond formation, available data indicate that the time scale of these two successive events are similar for L amino acids at physiological pH (Johansson et al. 2011, Juette et al. 2016). This implies that the kinetic constants of peptide bond formation (k_{pep}) for D-Phe and L-Phe are related by a similar factor of ~50-fold, which according to the structural contexts of the reaction (Fig. 1d), is the acceleration provided by the proton shuttle through the A76 2'OH.

A direct quantification of the effect of the A76 2'OH was obtained earlier by Green and coworkers. By subjecting aa-tRNA to aminolysis, where an attacking amine from either tris or hydroxylamine was used to break the ester bond of an aminoacyl-tRNA, they established that the reaction was ~10 times faster with a regular A76 2'OH as compared to a deoxy A76 2'H (Zaher et al. 2011). The acceleration provided by the proton shuttle on the ribosome, as estimated from the D/L amino acids experiments, is thus about 5 times faster. This is consistent with the suggestion that a water molecule stabilized at an appropriate location (Fig. 1d) is part of an improved proton exchange mechanism (Schmeing et al. 2005a), a possibility corroborated by analyses of kinetic solvent isotope effects (Kuhlenkoetter et al. 2011, Rodnina 2013). The acceleration of the rate of the reaction of peptide bond formation as achieved by the ribosome was found to be ~3 x 10⁷ (Sievers et al. 2004, Schroeder and Wolfenden 2007). It can thus readily be concluded that the ~50-fold shuttle effect associated with the A76 2'OH of the P-site tRNA is comparatively small. An accurate determination of this effect, required to support the present conclusion, should be achievable by establishing the kinetics of accommodation/peptide bond formation with L and D amino acids at a pH lower than 7.0, for which peptide bond formation is fully rate limiting (Johansson et al. 2011).

The A76 2'OH has yet another essential role in the reaction of peptide bond formation. It is involved in an induced-fit mechanism of the PTC that orients the amino group for nucleophilic attack (Zaher et al. 2011, Lehmann 2017). In solution, this group has an extended conformational freedom due to the flexibility of the ester bond connecting the amino acid to the tRNA, and studies on intramolecular reactions have shown that this feature severely affects the rate of a reaction (Bruice and Pandit 1960). On the ribosome, this impediment is resolved by a mechanism in which the binding of the tRNA 3' end at the entrance of the PTC triggers a large conformational change of the U2506 residue, that swings by about 90° to wedge the C_α of the aminoacyl ester against the A76 2'OH of the P-site tRNA (Fig. 1b). This action freezes the backbone of the amino acid while orienting its amino group for nucleophilic attack. Remarkably, this mechanism can handle both L and D enantiomers (Fig. 1b, d), although the amino group is pointing away from the A76 2'OH in the latter case (Lehmann 2017, Melnikov et al. 2019). The location of the cavity accommodating the side chains is also best suited for L amino acids. Discovered by Steitz and coworkers, the induced fit of the PTC was initially thought only to prevent a premature release of the protein by hydrolysis during elongation (Schmeing et al. 2005b).

Summing up the above, the A76 2'OH undisputedly plays a major role in peptide bond formation. Yet, as we have seen, investigations on the compatibility of D amino acids with translation have fortuitously revealed that its contribution as a mediator of a proton shuttle is rather small, and elongation could still work without this shuttle (Fig. 1a).

Besides desolvation, it appears that the only feature associated with the ribosome that is likely to accelerate the chemical step is a water molecule trapped in the PTC at an appropriate place, although the A76 residue is also involved in that case (Fig. 1d, Schmeing et al. 2005a, Polikanov et al. 2014). Why did this catalytic site not acquire more powerful tools to boost the reaction of peptide bond formation in the course of its evolution? We believe the answer lies in the difficulty of making the reaction just happen. To enter into a chemical reaction, reactive species must first and foremost be appropriately positioned with respect to each other. This is usually not an issue with enzymes because their substrates are generally simple. The ribosome, on the other hand, has to deal with a far more difficult problem: together with the tRNAs, it must juxtapose an aminoacyl and a nascent protein that are both characterized by high conformational degrees of freedom, and tend to adopt different conformations depending on the side chains. Just because it can achieve this performance, the PTC of modern ribosomes is an incredible evolutionary achievement. The task of precisely packing these substrates so that they can react is so monumental that the ribosome cannot always accomplish it completely by itself. When confronted with difficult substrates such as polyproline tracks, it has to recruit the eIF5A cofactor (Ef-P in bacteria) to help achieve the positioning of the peptidyl-tRNA (Doerfel et al. 2013, Ude et al. 2013). Recent works on eIF5A (Schuller et al. 2017, Pelechano and Alepuz 2017) further revealed that this cofactor alleviates stalling on many motifs besides polyproline tracks, and is also involved in translation termination. All these adjustments require time. Another bottleneck, proofreading, is yet another likely reason why ribosomes do not have a high catalytic power. Increasing the accuracy of translation requires a time delay between initial tRNA binding and peptide bond formation, that evolution implemented through Ef-Tu/GTP and tRNA accommodation (Thompson and Stone 1977, leong et al. 2016). Even if ribosomes performing faster chemistry could exist, this would not change the rate of translation, implying that they would not provide a phenotype that could be selected throughout evolution.

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Figure legend

Figure 1. Translation and peptide bond formation with D amino acids. **A)** Time-course experiments of peptide synthesis with single D-ser and L-ser inclusions, with L-ser-tRNA and D-ser-tRNA at 6 μM (at higher concentrations, the L-ser peptide is synthesized at a slightly higher rate). Adapted from Fujino et al. (2013). **B)** A-site configuration with D-ala-tRNA in the PTC (Lehmann, 2017), modeled from pdb files 2WDM and 2WDN (Voorhees et al. 2009). Two key atoms involved in the orientation of the amino group through the induced-fit mechanism are highlighted with VDW spheres. U2506 swings by about 90° (red arrow) upon tRNA 3' acceptor arm binding. The dashed line highlights the nucleophilic attack. **C)** PTC view of the X-ray structure from pdb file 6N9F (Melnikov et al. 2019) with ACCA-(D-Phe) on the A site of the ribosome (only the terminal fragment is shown). The $\text{NH}_2 - 2'\text{OH}$ distance is 4.2 Å. **D)** Superimposition of D-ser-tRNA and L-ser-tRNA configurations with L-fmet-tRNA on the P site, modeled from the pdb structure file 1VY6 (Polikanov et al. 2014). In the nucleophilic attack, both amino – carbonyl carbon distances are ~ 3.1 Å (yellow dotted lines). The amino group of the L enantiomer can bind to the A76 2'OH whereas that of the D enantiomer cannot. The proton shuttle mechanism involving the A76 2'OH and a water molecule (W) is highlighted with blue dotted lines.

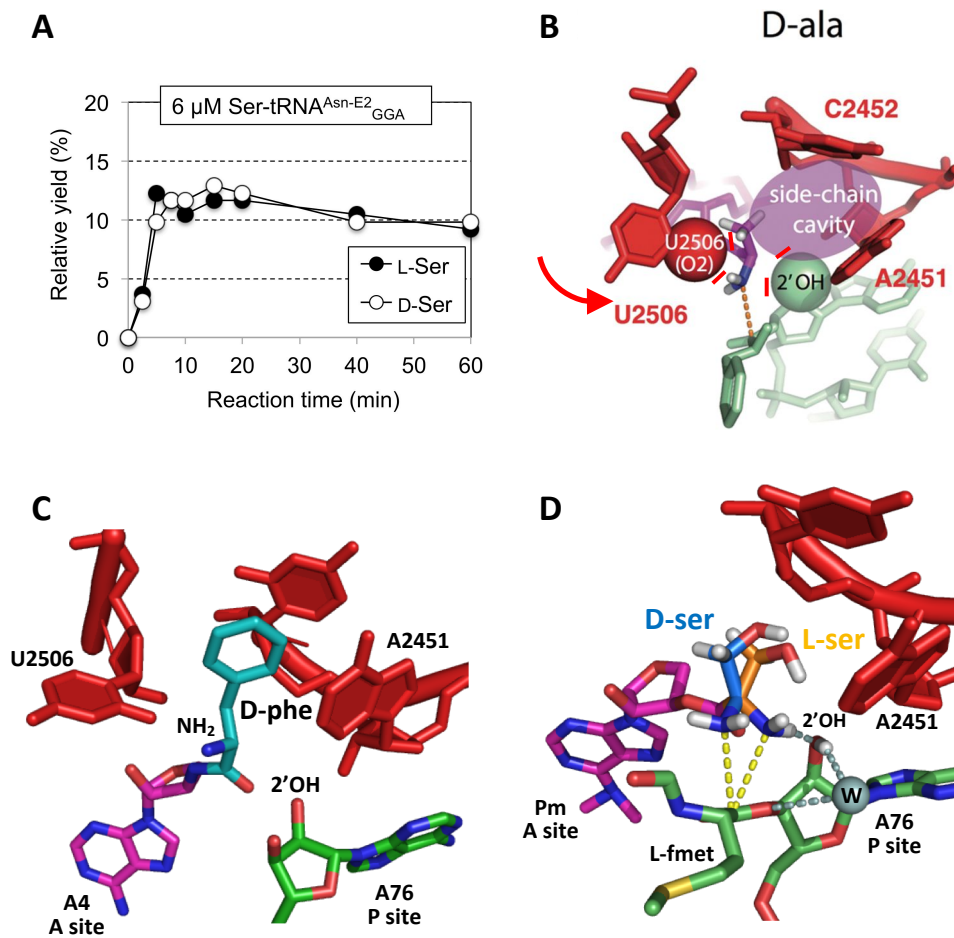


Figure 1