Minireview

D-Amino acids and D-Tyr-tRNA^{Tyr} deacylase: stereospecificity of the translation machine revisited

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Abstract Until 30 years ago, it had been considered that D-amino acids were excluded from living systems except for D-amino acids in the cell wall of microorganisms. However, D-amino acids, in the form of free amino acids, peptides and proteins, were recently detected in various living organisms from bacteria to mammals. The extensive distribution of bio-functional D-amino acids challenges the current concept of protein synthesis: more attention should be paid to the stereospecificity of the translation machine. Besides aminoacyl-tRNA synthetases, elongation factor Tu and some other mechanisms, D-Tyr-tRNA^{Tyr} deacylases provide a novel checkpoint since they specifically recycle misaminoacylated D-Tyr-tRNA^{Tyr} and some other D-aminoacyl-tRNAs. Their unique structure represents a new class of tRNA-dependent hydrolase. These unexpected findings have far-reaching implications for our understanding of protein synthesis and its origin.

Key words: D-Amino acid; D-Tyr-tRNA^{Tyr} deacylase; Stereospecificity; Protein synthesis

1. Introduction

High fidelity is crucial in the transmission and expression of genetic information. The identity of an amino acid inserted at a particular position in a nascent polypeptide is principally determined by two factors: the interaction of the aminoacyl-tRNA anticodon with an appropriate codon in mRNA and the correct pairing of amino acid and tRNA anticodon in the aminoacyl-tRNA [1–3]. Numerous quality control mechanisms have been demonstrated based on the concept that the ribosomally mediated protein synthesis is confined to the L-amino acid pool [4].

Thanks to the technological advances in chiral separation, extensive distribution of bio-functional D-amino acids in living organisms has been demonstrated in recent years [5–7]. Bioactive peptides and proteins with D-amino acid residues challenge our previous understanding of the homochirality of life. Because there is no special genetic codon for D-amino acids [8], aminoacyl-tRNA synthetases now seem to have to recognize their substrates from a much more complexed pool of amino acids with enantiomers. Meanwhile D-amino acids should be restricted to the minimum extent in protein synthesis since most of the proteins made up of a mixture of D- and L-amino acids would not be efficient because they cannot fold into bioactive configurations such as the α-helix [9]. Thus mechanisms for the stereospecificity of the translation machine are placed on the agenda. Here, we will review recent advances in our understanding of D-amino acids, and D-Tyr-tRNA^{Tyr} deacylases – currently known to be a novel proofreading mechanism in protein synthesis.

2. Distribution of D-amino acids in living organisms

One of the most amazing biological discoveries in recent years is the detection of D-amino acids in various living organisms (Table 1). They are much more functionally active than previously thought. Unicellular microorganisms produce, metabolize and utilize D-amino acids [10]. The D-amino acids in the bacterial walls contribute to their resistance to digestion by proteolytic enzymes. Certain antibiotics produced by bacteria and fungi contain D-amino acids, such as D-Asp, D-Trp, D-Val and D-Cys [6]. Free D-amino acids in marine species could be involved in osmoregulation and serve as a nutritional source of L-isomers [11], while in mammals free D-amino acids are related to developmental stage [12–14], hormone synthesis [15] and neurotransmission [23]. Small peptides containing one D-amino acid have been isolated from both vertebrates and invertebrates, including frog skin dermorphins [16], deltorphins [17], and peptides isolated from an African giant snail [18]. Furthermore, proteins containing D-amino acids have also been found in aged human tissues, such as eye lens crystallins [19], myelin basic protein [20], erythrocyte proteins [21], and β-amyloid peptides from Alzheimer disease brains [22].

The possible origins of D-amino acids in vivo are considered in three main aspects. (i) Free D-amino acids could be taken up from the environment. (ii) Some organisms could synthesize free D-amino acids de novo. For example, human serine racemase catalyzes the formation of D-serine from L-serine. Robust synthesis of D-serine was detected in cells transfected with human serine racemase, demonstrating the conservation of D-amino acid metabolism in humans [23]. (iii) The D-amino acid residues in peptides and proteins are considered to be the result of slow posttranslational epimerization process [8,24]. One of the examples is the functional consequences of post-translational isomerization of Ser46 in a calcium channel tox-

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Table 1
D-Amino acids in living organisms

D-Amino acid	Organism	Type	Physical meaning
D-Ala	rice	peptide	unknown
D-Ala, D-Asp	phytoplankton	free	unknown
D-Ala, D-Glu	bacteria	peptide	peptidoglycans
D-Thr, D-Ser	bacteria	peptide	peptidoglycans
D-Asp, D-Trp	bacteria	peptide	antibiotics
D-Cys, D-Leu	bacteria	peptide	antibiotics
D-Val, D-Orn	bacteria	peptide	antibiotics
D-Ala, D-Asp	fungi	compound	pigments
D-Ser	spider	peptide	neuropeptides
D-Phe, D-Asn	snail	peptide	neuropeptides
D-Ala	marine invertebrates	free	osmoregulation
D-Ala, D-Met	frog	peptide	opioid peptides
D-Asp, D-Ser	human, mouse, rat	free	development
D-Asp	human, cattle, mouse, rat	protein	aging
D-Ser	human	protein	aging
D-Ser	human	free	neurotransmitter

Revised from [7].

in of the venom of the funnel-web spider *Agelenopsis aperta* [25]. A cofactor-independent serine isomerase in the venom of *A. aperta* was found to be capable of isomerizing serine, cysteine, *O*-methylserine and alanine in the middle of peptide chains [26].

No matter which origin they come from, these unusual molecules play a minor but undeniable part in the orchestra of life. The immediate question following this phenomenon is that the translation machine has to discriminate D-amino acids in a more accurate way.

3. The problem of D/L-amino acid discrimination

Protein synthesis is a highly accurate process: usually only 1 in every 10 000 codons in mRNA is encoded incorrectly [27]. Numerous proofreading mechanisms target almost every step of the transmission and expression of the genetic code. Since amino acids are provided in the form of aminoacyl-tRNAs, at least three aspects are aimed at D-aminoacyl-tRNAs: (i) to disturb the formation of the ester linkage between D-amino acids and tRNAs; (ii) to hydrolyze the ester linkage between D-amino acids and tRNAs; (iii) to prevent D-aminoacyl-tRNAs binding to the ribosome's A-site by elongation factor Tu [28,29].

Aminoacyl-tRNA synthetases play a central role in maintaining accuracy during the translation of the genetic code. To achieve this challenging task, they have to discriminate against amino acids that are very closely related not only in structure but also in chemical nature. D-Amino acids are very similar to their L-isomers except for the polarizing optic character. However, the stereospecificity of aminoacyl-tRNA synthetases is not unconditional, although most of them can adequately discriminate D- from L-amino acids [1]. It was observed early on that *Escherichia coli* and *Bacillus subtilis* tyrosyl-tRNA synthetases can transfer D-tyrosine to $tRNA^{Tyr}$. The same extent of $tRNA^{Tyr}$ aminoacylation could be reached with the L- and the D-enantiomers of the amino acid [30,31]. D-Valine binding to valyl-tRNA Val synthetase was reported by Owens and Bell [32,33]. In the work of Soutourina et al., production of D-Asp-tRNA Asp and D-Trp-tRNA Trp by aspartyl-tRNA synthetase and tryptophanyl-tRNA synthetase, respectively, was established in vitro [34].

Although a few aminoacyl-tRNA synthetases, including valyl-tRNA synthetase, have the capacity to promote hydrolysis of their misproducts through efficient proofreading mechanisms [35], the toxicity of D-aminoacyl-tRNAs to the growth of *E. coli* and *Saccharomyces cerevisiae* suggests that D-aminoacyl-tRNA molecules could be present in vivo under given conditions [34,36]. Then the immediate question is that there must be specific measures to get rid of D-aminoacyl-tRNAs.

4. D-Tyr-$tRNA^{Tyr}$ deacylases: unique structure and characteristic function

It was first observed by Calendar and Berg that extracts of *E. coli*, yeast, rabbit reticulocytes, and rat liver contained an enzyme activity capable of accelerating the hydrolysis of the ester linkage of D-Tyr-$tRNA^{Tyr}$ in the production of free tRNA and D-tyrosine [37]. Partially purified *E. coli* deacylase could be shown to be distinct from tyrosyl-tRNA synthetase [37] or peptidyl-tRNA hydrolase [38]. Soutourina et al. first identified D-Tyr-$tRNA^{Tyr}$ deacylase in *E. coli* [39] and yeast [34]. The identification of D-Tyr-$tRNA^{Tyr}$ deacylase in several

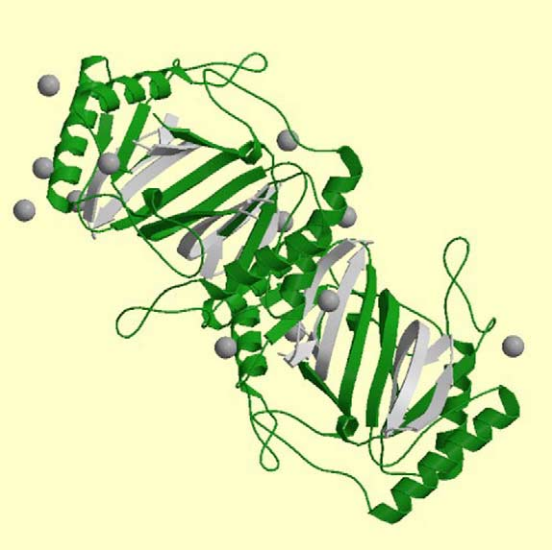

Fig. 1. Crystal structure of D-Tyr-$tRNA^{Tyr}$ deacylase of *E. coli* (see [40] for references). PDB accession number 1JKE.

organisms provides a novel checkpoint for the proper production of L-aminoacyl-tRNAs.

D-Tyr-tRNA^{Tyr} deacylase is established to be an editing enzyme that removes D-tyrosine and other D-amino acids from charged tRNAs, thereby preventing incorrect incorporation of D-amino acids into proteins. *E. coli* D-Tyr-tRNA^{Tyr} deacylase is encoded by the *dtd* gene [39]. Orthologs of the *dtd* gene occur in many bacteria as well as in *S. cerevisiae*, *Caenorhabditis elegans*, *Arabidopsis thaliana*, mouse and human. Involvement of the deacylase in the protection against in vivo produced D-Tyr-, D-Trp- or D-Asp-tRNA is demonstrated by the exacerbation of the toxicity of each of these three D-amino acids in response to the inactivation of the *E. coli dtd* gene [34,39]. The toxic effects of D-Gln and D-Ser also respond to the absence of the *dtd* product [34]. In the case of yeast, inactivation of the DTD1 gene also leads to the toxicity of D-Tyr and D-Leu [36]. While the enzyme exhibits broad specificity towards D-amino acids, it is inactive towards L-aminoacyl-tRNAs and *N*-blocked D-aminoacyl-tRNAs [39]. It looks like D-Tyr-tRNA^{Tyr} deacylases recognize almost all D-aminoacyl-tRNAs instead of only D-Tyr-tRNA^{Tyr}, so it would be better to call them D-aminoacyl-tRNA deacylases.

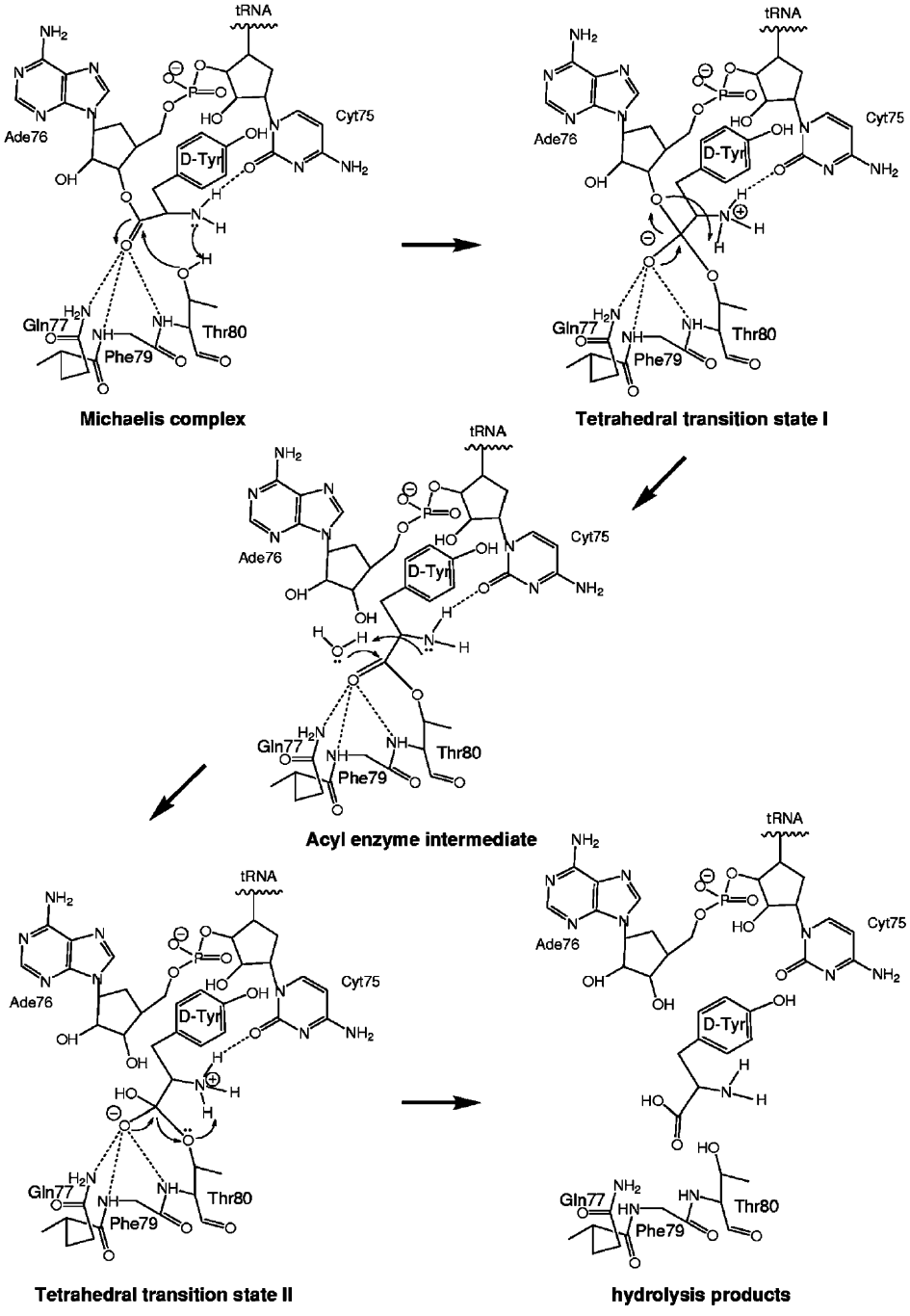

Fig. 2. A scheme of the proposed catalytic mechanism of D-Tyr-tRNA^{Tyr} deacylase deduced by docking a model of D-Tyr-tRNA^{Tyr} in the proposed enzyme active site (see [39] for references).

The solved structure of D-Tyr-tRNA^{Tyr} deacylase differs markedly from those of all other documented tRNA-dependent hydrolases. Their dimeric structure corresponds to a β-barrel closed on one side by a β-sheet lid [40]. Each monomer contains a five-stranded mixed twisted β-sheet, and a three-stranded antiparallel β-sheet (Fig. 1). While Ferri-Fioni and colleagues list structural similarities between portions of the molecules and segments of other proteins [40], Lim et al. considered the structure to be unique to D-Tyr-tRNA^{Tyr} deacylase [41] based on Holm and Sander [42].

A Michaelis complex and catalytic mechanism (Fig. 2) were proposed based on the crystal structure of *Haemophilus influenzae* HI0670 [41]: In the deacylase, Thr80 hydroxyl serves as the nucleophilic group, there is an oxyanion hole to stabilize the negatively charged tetrahedral transition state, and the reaction is substrate assisted by the amino group of D-Tyr providing a 'proton park' for the Thr80 hydroxyl group.

However, none of the publications so far established the exact catalytic mechanism. The deacylase possibly recognizes a feature common to all tRNAs, for instance the CCA triplet at the end of the acceptor stem of the polynucleotide. On the side of the amino acid moiety, the deacylase would only distinguish the stereoisomeric character of the Cα [39]. The crystal structure of the enzyme–tRNA complex will reveal the exact interactions in the active site, and validate or refute the proposed mechanisms.

5. Conclusion and perspectives

Although extensive physical, chemical and biological mechanisms have been proposed to explain the pervasive amino acid homochirality of protein synthesis, the origins of this phenomenon have never been explained satisfactorily. With the detection of the wide distribution of D-amino acids in living organisms, it is much more imperative for accurate translation that tRNAs are only coupled to L-amino acids corresponding to the RNA anticodon. D-Tyr-tRNA^{Tyr} deacylases specifically hydrolyze D-Tyr-tRNA^{Tyr} and other D-aminoacyl-tRNAs, thus provide another checkpoint for the stereospecificity of the translation machine. These unexpected findings have far-reaching implications for our understanding of the stereospecificity of protein synthesis.

More functional characterization should be done on D-Tyr-tRNA^{Tyr} deacylases in other species such as mouse and human. With the characterization of the crystal structure of the enzyme–tRNA complex, it will be very interesting for us to understand the exact way in which the deacylase recognizes those unconventional molecules.

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