
Insights Into the Peptide Bond Formation from a Mechanistic Perspective

During protein synthesis, ribosomes are considered responsible for discriminating between the two chiral forms of amino acids and preventing the integration of D-amino acids into nascent proteins by reducing the rate at which [peptide bonds](#) are formed. Even though this phenomenon has been recognised for nearly forty years, no structures that might account for the low reactivity of D-amino acids have ever been discovered.

Researchers describe a crystal structure of a bacterial ribosome in association with a D-aminoacyl-tRNA analogue coupled to the A site. The resolution of the crystal structure is 3.7 angstroms. At this resolution, researchers could not see individual chemical groups. Still, based on the chemical constraints, they could unequivocally establish the locations of the D-amino acid side chain and the amino group. The structure indicated that the D-amino acid, much like the L-amino acid, attaches to the ribosome by inserting its side chain into the ribosomal A-site cleft. This is the same mechanism that is used by L-amino acids. Due to the nature of this binding mechanism, an optimum nucleophilic assault on the peptidyl-tRNA by the reactive-amino group of a D-amino acid is not possible. In addition, the uncovered structure leads specialists to believe that the D-amino acid cannot make hydrogen bonds with the P-site tRNA, which are essential for the successful transfer of proton during the creation of peptide bonds. In general, the research results provide the first mechanistic insight into an aging process that aids live cells in ensuring the stereochemistry of protein production.

L- and D-amino acids are found in equal amounts in all living cell types. On the other hand, the production of proteins is considered to require simply the use of L-amino acids. The mechanism behind this particular use of just L-amino acids is not yet completely understood. This is particularly noteworthy in bacteria since their cytosols include over a dozen distinct D-amino acids that may serve as a carbon source, signalling molecules or building blocks for creating peptidoglycan cell wall components [i],[ii]. Some bacteria have millimolar concentrations of D-amino acids, and these millimolar concentrations may occasionally have levels of D-isomers that are higher than those of their L-isomers (as is the case with D-alanine and D-glutamate) [ii],[iii]. Nanomolar to micromolar amounts of D-amino acids are generally found in eukaryotic organisms such as animal, plant, and fungal cells [iv],[v]. Despite D-amino acids in the cell's cytoplasm, only L-amino acids can be used by organisms to synthesise proteins. This holds for bacteria as well as higher eukaryotic species.

At least four mechanisms are considered to work together to prevent D-amino acids from being used in ribosome-dependent protein synthesis. This is accomplished via the collaboration of several systems. To begin, the enzymes known as aminoacyl-tRNA-synthetases, responsible for selecting amino acids for protein synthesis, respond noticeably more slowly with D-amino acids than with L-amino acids. For example, the rate at which tyrosyl-tRNA synthetase uses D-tyrosine to create tyrosyl-tRNA is about twenty-five times slower than the rate at which it uses L-tyrosine [vi]. Second, if D-aminoacyl-tRNAs are created, they are normally deacylated by an enzyme called D-aminoacyl-tRNA deacetylase (DTD) [vii],[viii]. This enzyme, conserved throughout all three domains of life, is responsible for preventing the build-up of D-aminoacyl-tRNAs and the toxicity they cause [ix]. Third, if a D-aminoacyl-tRNA can avoid being hydrolysed by DTD, then the elongation factor EF-Tu can detect it.

On the other hand, its distribution to the ribosome happens with a yield about 250 times lower than that of L-aminoacyl-tRNAs [x]. Finally, in vitro tests suggest that if a D-aminoacyl-tRNA binds the ribosomal A site, it interacts with a P-site substrate about three orders of magnitude slower than the rate at which it reacts with L-aminoacyl-tRNAs. This demonstrates that D-amino acids significantly slow the rate at which peptide bonds are formed [xi]. A D-amino acid can induce translation arrest if integrated into a nascent peptide and then translocated to the P site. This suggests that D-amino acids also interfere with the transit of the nascent peptide via the ribosomal exit tunnel [xi]. Therefore, cells contain complex fidelity control mechanisms that encourage the preferred employment of the L-isomers over the D-amino acids at every step of protein synthesis. These systems provide preference to the L-isomers over the D-amino acids.

By manipulating various components of the translation machinery over the last several years, it has been feasible for scientists to perform the messenger RNA-dependent synthesis of D-amino acid-containing proteins. For instance, protein engineering has made it possible to create aminoacyl-tRNA synthetases that exclusively employ the D-isomer of the amino acid tyrosine [xii],[xiii]. Additionally, the generation of D-aminoacyl-tRNAs that may be used in cell-free protein translation systems became conceivable due to the creation of designed catalytic RNAs known as flexizymes [xiv]. Synthesis of measurable quantities of peptides, including up to 10 consecutive D-amino acids, was made possible because of the optimisation of translation systems. More advancements were made through a process known as random mutagenesis of ribosomal RNA (rRNA) [xv],[xvi].

For example, ribosomes that had mutations in the nucleotides located at 2447GAUA2450 in the 23S rRNA showed a significant increase in their compatibility with D-amino acids. However, these ribosome mutants were useless in *Escherichia coli* because they were less accurate and toxic.

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