

Pyrrolysine

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summary

Pyrrolysine (Pyl) is recognized as the 22nd genetically encoded amino acid, distinguished by its unique chemical structure that resembles lysine but features a 4-methylpyrroline-5-carboxylate moiety linked to its ϵ -amino group. This rare amino acid plays a crucial role in certain methanogenic archaea and bacteria, where it is essential for the enzymatic transfer of methyl groups in the process of methano-

genesis, contributing significantly to the global carbon cycle.[\[1\]\[2\]\[3\]](#) Pyrrolysine's incorporation into proteins is mediated by specialized machinery that repurposes the UAG stop codon, underscoring its distinct position in the genetic code and evolutionary biology.[\[4\]\[3\]](#)

The biosynthesis of pyrrolysine occurs through a specialized pathway involving the radical S-adenosylmethionine (SAM) enzyme, which modifies lysine into pyrrolysine. This pathway is catalyzed by a series of enzymes, including PylB, PylC, and PylD, and is encoded within the *pylSTBCD* operon.[\[5\]\[6\]\[7\]](#) Researchers have been intrigued by the evolutionary implications of pyrrolysine, as it raises questions about the mechanisms behind the incorporation of non-canonical amino acids into genetic codes across different organisms.[\[8\]\[9\]](#)

Pyrrolysine's distinct properties have led to its applications in synthetic biology and biotechnology, where it facilitates the incorporation of non-standard amino acids into proteins, thereby enabling novel biochemical functions.[\[4\]\[10\]](#) Its potential for therapeutic development is particularly noteworthy, as pyrrolysine's site-specific incorporation into proteins allows for the design of advanced drug delivery systems and targeted therapies, particularly in the context of cancer treatment.[\[11\]\[10\]](#)

Despite its significant roles and applications, pyrrolysine remains a topic of ongoing research, particularly regarding its biochemical pathways, evolutionary origins, and potential functions beyond methanogenesis. As the study of this amino acid progresses, it promises to enhance our understanding of amino acid diversity and the complexities of protein synthesis in living systems.[\[8\]\[12\]\[13\]](#)

Chemical Structure

Pyrrolysine (Pyl) is a unique amino acid that exists as the 22nd proteinogenic amino acid, notable for its distinct chemical structure and properties. It is classified as a modified form of lysine, featuring a 4-methylpyrroline-5-carboxylate group that is linked by an amide bond to the α -amino group of lysine, creating the structure of L-pyrrolysine[\[1\]\[14\]](#).

Asymmetric Unit in Crystallography

In crystallographic studies, the asymmetric unit contains two molecules of S^H-PylRS (pyrrolysyl-tRNA synthetase) and two molecules of S-DhtRNAPyl (pyrrolysine-specific tRNA). This structural configuration provides insights into the specific interactions and functions of the pyrrolysine system, contributing to our understanding of how this amino acid is incorporated into proteins[\[15\]\[1\]](#).

Properties of Pyrrolysine

Pyrrolysine exhibits a remarkably distinct structure compared to standard amino acids, particularly due to its reactive electrophilic moiety, which plays a crucial role in various biochemical processes, including methyl group transfer reactions in methyltransferases[\[2\]\[1\]](#). This characteristic is pivotal for its function in certain

methanogenic archaea and bacteria, where it participates in the enzymatic transfer of methyl groups from substrates such as trimethylamine to coenzyme M[16][17].

Biosynthesis

Pyrrolysine (Pyl), recognized as the twenty-second amino acid encoded by the natural genetic code, is synthesized through a specific biosynthetic pathway involving the essential amino acid lysine as its sole precursor[5][6]. The process involves the condensation of two lysine molecules facilitated by three key enzymes: the radical S-adenosylmethionine (SAM) enzyme PylB, ATP-dependent PylC, and PylD[1][18].

Enzymatic Steps

The initial step in Pyl biosynthesis is catalyzed by PylB, which converts lysine into (3R)-3-methyl-d-ornithine[19]. This reaction sets the stage for subsequent enzymatic modifications that ultimately lead to the formation of pyrrolysine. The complete pathway, which integrates rational engineering and directed evolution, has been proposed to improve the efficiency of Pyl production in various organisms, including *E. coli*, where the components of this pathway, particularly PylB, are often expressed suboptimally[7][15].

Genetic Components

The genetic information necessary for the biosynthesis and incorporation of pyrrolysine into proteins is encoded within a single operon known as pylSTBCD. This operon orchestrates the synthesis of Pyl and its incorporation into nascent polypeptides[1][5]. The functionality of this system demonstrates the potential for expanding the genetic code by introducing noncanonical amino acids into living cells, thus providing insights into amino acid evolution and the mechanisms behind the development of new biosynthetic pathways[7][8].

Implications for Research

Understanding the biosynthesis of pyrrolysine not only elucidates the chemical feasibility of its pathway but also opens avenues for enzymologists and geneticists to explore how amino acids are integrated into the genetic code throughout evolution[8][5]. Researchers are encouraged to apply insights from this naturally occurring biosynthetic pathway to develop innovative strategies for the synthesis and utilization of noncanonical amino acids in various biotechnological applications[7].

Biological Role

Pyrrolysine, known as the 22nd genetically encoded amino acid, plays a crucial role in certain organisms, particularly in methanogens such as *Methanocaldococcus jannaschii*, where it is involved in the process of methanogenesis, the biological production of methane.[2][3]. Its structure closely resembles lysine, and it aids the enzyme methyltransferase during

this metabolic process, facilitating the conversion of substrates into methane, which is a significant part of the global carbon cycle[\[3\]](#).

In addition to its role in methanogenesis, pyrrolysine is considered a rare amino acid due to its limited occurrence in nature. While it has primarily been found in methanogens, further research is ongoing to explore any potential additional functions it may have in other biological contexts[\[3\]](#). The evolutionary origins of pyrrolysine, like selenocysteine, remain somewhat enigmatic, raising questions about the circumstances under which these non-canonical amino acids were incorporated into the genetic codes of certain organisms[\[3\]\[9\]](#).

The study of pyrrolysine not only enhances our understanding of amino acid diversity but also emphasizes the complexities of protein synthesis and function in different life forms. As researchers continue to investigate its biochemical roles, the significance of pyrrolysine in the broader spectrum of biological systems may become clearer, potentially revealing more about the evolutionary adaptations of certain species[\[3\]\[9\]](#).

Applications

Pyrrolysine, recognized as the 22nd genetically encoded amino acid, has diverse applications in the field of synthetic biology and biotechnology. Its unique properties allow for the incorporation of non-standard amino acids into proteins, facilitating novel biochemical functions and therapeutic strategies.

Protein Engineering

One of the primary applications of pyrrolysine is in the engineering of proteins to enhance their functionalities. By incorporating pyrrolysine into proteins, researchers can modify their properties for specific applications, such as improved stability, activity, or selectivity in enzymatic reactions[\[4\]\[11\]](#). For instance, engineered pyrrolysyl-tRNA synthetases enable the incorporation of pyrrolysine at specific sites within proteins, allowing for the study of protein interactions and functions in greater detail[\[4\]\[10\]](#).

Therapeutic Developments

Pyrrolysine has promising potential in therapeutic applications, particularly in the design of covalent protein drugs and targeted therapies. Its ability to be site-specifically incorporated into proteins allows for the development of advanced therapeutic agents with improved efficacy and reduced side effects. Recent studies have demonstrated the successful use of pyrrolysine in developing reactive therapeutics that can selectively target diseased cells, thus paving the way for more precise treatment options in cancer therapy and other diseases[\[4\]\[10\]\[11\]](#).

Research and Diagnostic Tools

In addition to therapeutic uses, pyrrolysine is increasingly employed in research settings as a tool for studying protein function and dynamics. Its unique incorporation into proteins can be utilized in various assays and imaging techniques to track protein

localization and interactions within live cells[\[4\]\[11\]](#). This capability enhances our understanding of cellular processes and the role of specific proteins in health and disease.

Expansion of the Genetic Code

The incorporation of pyrrolysine into the genetic code represents a significant advancement in synthetic biology, allowing scientists to explore the boundaries of the genetic code and its applications. Researchers are utilizing this expanded genetic code to create novel proteins with non-canonical amino acids, which can have unique physical and chemical properties not found in naturally occurring proteins[\[4\]\[10\]](#). This has implications not only for fundamental biological research but also for the development of new materials and biocatalysts.

Current Research

Pyrrolysine, recognized as the 22nd genetically encoded amino acid, is of significant interest in contemporary biochemical research due to its unique synthesis and functional roles in archaea and bacteria[\[2\]\[20\]](#). Recent studies have focused on the biosynthetic pathways that facilitate the incorporation of pyrrolysine into proteins. Notably, it is synthesized in vivo through the ligation of two molecules of L-lysine, which undergoes several enzymatic modifications including conversion to (3R)-3-methyl-D-ornithine[\[21\]\[22\]](#).

Biochemical Significance

Research has highlighted the importance of pyrrolysine in energy conversion processes within methanogens, a type of archaea that thrive in anaerobic conditions[\[23\]\[20\]](#). The distinct role of pyrrolysine in the translation machinery of these organisms has prompted investigations into the evolutionary aspects of its incorporation into the genetic code. It has been suggested that understanding the biosynthetic pathways of non-standard amino acids like pyrrolysine can provide insights into how amino acids evolve and diversify within different organisms[\[8\]\[22\]](#).

Advances in Genetic Engineering

Efforts in genetic engineering have aimed to exploit pyrrolysine's properties for applications in synthetic biology. For instance, research has focused on engineering tRNA and aminoacyl-tRNA synthetases to enhance the incorporation efficiency of pyrrolysine and other noncanonical amino acids in various cellular systems[\[4\]\[21\]](#). This has potential implications for the development of novel biocatalysts and therapeutics, broadening the scope of amino acid utilization in protein engineering[\[5\]](#).

Future Directions

The ongoing exploration of pyrrolysine not only aims to elucidate its biochemical pathways but also seeks to enhance our understanding of the broader implications

of amino acid diversity in biological systems. Continued research is expected to investigate the intricate mechanisms by which pyrrolysine is incorporated into proteins and its functional ramifications within various metabolic contexts[12][13]. As scientists look to nature for inspiration, the simplicity and elegance of pyrrolysine's biosynthetic pathway may pave the way for innovative designs in the field of amino acid synthesis and genetic code expansion[8].

History

Pyrrolysine was first identified as a unique amino acid in the early 2000s, specifically in the context of methanogenic archaea. The first comprehensive description of the mechanism by which pyrrolysine is incorporated into proteins was published in 2004 by researchers led by Blight, who elucidated the molecular processes involved in its incorporation through specialized tRNA and synthetases[4]. Subsequent studies confirmed that pyrrolysine is encoded by the UAG stop codon, which is repurposed for its incorporation in certain archaeal species[4][3].

In 2011, a significant review by Krzycki and colleagues detailed the functional context and biosynthesis of pyrrolysine, highlighting its genetic encoding and emphasizing its role in the metabolism of methanogenic microorganisms[4]. The advancements in understanding pyrrolysine have led to its application in synthetic biology, where engineered aminoacyl-tRNA synthetases and tRNA pairs are used to expand the genetic code, allowing the incorporation of pyrrolysine into proteins in vitro and in vivo[4][21].

The engineering of pyrrolysyl-tRNA synthetases has paved the way for utilizing pyrrolysine in a broader range of biological and biochemical applications. Research has demonstrated its potential for facilitating the incorporation of non-canonical amino acids into proteins, thus enabling the exploration of new protein functions and properties[21][24]. As the study of pyrrolysine continues, it plays a crucial role in advancing our understanding of genetic code expansion and protein engineering[3][25].

References

- [1]: [Improved pyrrolysine biosynthesis through phage assisted non ...](#)
- [2]: [Pyrrolysine - an overview | ScienceDirect Topics](#)
- [3]: [Engineering Pyrrolysine Systems for Genetic Code Expansion and ...](#)
- [4]: [Insights into pyrrolysine function from structures of a trimethylamine ...](#)
- [5]: [EC 6.3.2.59 - IUBMB Nomenclature](#)
- [6]: [How does the MPEP define standard amino acids? - BlueIron IP](#)
- [7]: [The complete biosynthesis of the genetically encoded amino acid ...](#)
- [8]: [2.2: Structure & Function - Amino Acids - Biology LibreTexts](#)
- [9]: [How two amino acids become one - PMC](#)
- [10]: [The path of lysine to pyrrolysine - ScienceDirect.com](#)
- [11]: [Pyrrolysine-Inspired in Cellulo Synthesis of an Unnatural Amino ...](#)

- [12]: [Pyrrolysine-Synthesis-Revealed - C&EN - American Chemical Society](#)
- [13]: [What Are The Two Rare Amino Acids? - ScienceABC](#)
- [14]: [40 Lysine Foods and Supplements, Plus Benefits and Risks](#)
- [15]: [Pyrrolysine Aminoacyl-tRNA Synthetase as a Tool for Expanding the ...](#)
- [16]: [L-Pyrrolysine | CAS NO.:448235-52-7 - GlpBio](#)
- [17]: [CHEBI:21860 - L-pyrrolysine - EMBL-EBI](#)
- [18]: [Pyrrolysine - an overview | ScienceDirect Topics](#)
- [19]: [Pyrrolysine - Wikipedia](#)
- [20]: [The pyrrolysine translational machinery as a genetic-code ...](#)
- [21]: [What makes Selenocysteine and Pyrrolysine different than the rest ...](#)
- [22]: [The Pyrrolysine Translational Machinery as a Genetic-Code ...](#)
- [23]: [The complete biosynthesis of the genetically encoded amino acid ...](#)
- [24]: [Scientists Unlock Mystery Of How The 22nd Amino Acid Is Produced](#)
- [25]: [Discovering the 21st Amino Acid ... Again? - The Scientist](#)