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Review

Enantiomeric separation of nonproteinogenic amino acids by high-performance liquid chromatography

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ABSTRACT

Amino acids are essential for life, and have many functions in metabolism. One particularly important function is to serve as the building blocks of peptides and proteins, giving rise complex three dimensional structures through disulfide bonds or crosslinked amino acids. Peptides are frequently cyclic and contain proteinogenic as well as nonproteinogenic amino acids in many instances. Since most of the amino acids contain a chiral carbon atom, the stereoisomers of all these amino acids and the peptides in which they are to be found may possess differences in biological activity in living systems. The development of methods for the separation of enantiomers has attracted great interest, since it became evident that the potential biological or pharmacological applications are mostly restricted to one of the enantiomers. The important analytical task of the separation of isomers is achieved mainly by chromatographic and electrophoretic methods. This special review surveys indirect and direct high-performance liquid chromatographic (HPLC) methods of biologically and pharmaceutically important enantiomers of nonproteinogenic amino acids and related compounds, with emphasis on the literature published from the beginning.

Contents

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1. Introduction

Since the discovery of optical isomerism by Pasteur [1], the importance of chirality with respect to biological activity has been clearly recognized. The physiological environment within a living organism is chiral, and the biological activities of enantiomeric forms of molecules can differ dramatically. The biological or pharmacological activities of compounds are mostly restricted to one of the enantiomers. The differences in biological properties of enantiomers arise from the differences in protein transport and binding, the kinetics of their metabolism and disappearance and their stability in the environment [2,3]. The pharmacologically inactive enantiomer can exhibit unwanted side effects, antagonistic activities or even may be toxic. Even if these side effects are not drastic, the unwanted enantiomer has to be metabolized in the organism and represents an unnecessary burden for the organism.

With the exception of Gly, all encoded proteinogenic amino acids have at least one chiral center (enantiomers) or even two (epimers). Some 20 genetically encoded amino acids comprise the building blocks of proteins, which besides nucleotides, polysaccharides or lipids are the most important constituents of all living systems. Proteins of multicellular organisms are usually based on L-amino acids but the D-forms of amino acids in peptides can differ significantly in biological systems [4–7].

The large numbers of nonproteinogenic amino acids found in nature occur in free form or as simple condensation products such as γ-glutamyl, acetyl, and oxalyl derivatives and as constituents of other biomolecules such as peptides, proteins, coenzymes, and hormones.

The nonproteinogenic amino acids are found mostly in plants and microorganisms and arise as intermediates or as the end-products of metabolic pathways. They may also arise in the process of detoxification of compounds of foreign origin. Nonproteinogenic amino acids play an important role in the food and pharmaceutical industries and are useful as building blocks for the synthesis of analogs of biologically active peptides, antibiotics, hormones, and enzyme inhibitors [8]. They are also versatile chiral starting materials or chiral auxiliaries in many organic syntheses [9]. Several hundreds of such amino acids are known, and a large number of these are α- and β-amino acids.

1.1. Natural occurrence and biological activity of nonproteinogenic amino acids

There are several examples for natural occurrence and biological activity of nonproteinogenic amino acids but for most of them it is difficult to ascribe an obvious direct function in the organism. 1-Aminocyclopropane-1-carboxylic acid is a key intermediate in the production of the plant hormone ethylene, it is synthesized by the enzyme ACC synthase from Met and converted to ethylene by ACC oxidase [10]. L-Azetidine-2-carboxylic acid, a homolog of Pro, and orcylalanine may be considered as a substituted Phe or Tyr. It is known to occur in the flowering plant family *Ruscaceae* and is also found in numerous plants from the family *Fabaceae* [11]. *N*-acetyl-L-Tyr is formed in the metabolism of Tyr and serves as an efficient supplement for raising Tyr levels in the body. Vitamin B_6, a required cofactor for neurotransmitter synthesis also contains *N*-acetyl-L-Tyr. *N*-acetylserotonin methyltransferase enzyme activity was inhibited by various related compounds such as *N*-acetyltryptamine and *N*-acetyl-L-Tyr [12,13]. Betaine is involved in Gly biosynthesis and is a methyl donor of increasing significance. It is widely distributed in microorganisms, plants and animals [14]. L-(+)-(*S*)-Canavanine, structurally related to the L-Arg, is found in certain leguminous plants. Canavanine is accumulated primarily in the seeds of the organisms which produce it, where it serves both as a highly deleterious defensive compound against herbivores and a vital source of nitrogen for the growing embryo [15]. Carnitine is a quaternary ammonium compound biosynthesized from the amino acids Lys (*via* trimethyllysine) and Met [16]. Vitamin C is essential to the synthesis of carnitine. In living cells, it is required for the transport of fatty acids from the cytosol into the mitochondria during the breakdown of lipids for the generation of metabolic energy. Citrulline is made from ornithine (Orn) and carbamoyl phosphate in one of the central reactions in the urea cycle. It is also produced from Arg. Proteins that contain citrulline residues include myelin basic protein (MBP), filaggrin, and several histone proteins, whereas other proteins, such as fibrin and vimentin are susceptible to citrullination during cell death and tissue inflammation [17]. Creatine is a nitrogenous organic acid that occurs naturally in vertebrates and helps to supply energy to all cells in the body, primarily muscle. It is naturally produced in the human body from L-Arg, Gly, and L-Met primarily in the kidney and liver [18]. Levodopa (L-DOPA) is produced from the amino acid L-Tyr by the enzyme *tyrosine hydroxylase* (TH). It is also the precursor for the monoamine or catecholamine neurotransmitters dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline) [19]. Lanthionine is a sulfide-bridged Ala dimer, is found widely in nature and have been isolated from human hair, lactalbumin, and feathers. Lanthionines have also been found in bacterial cell walls and are the components of a group of gene encoded peptide antibiotics called lantibiotics, which includes nisin, subtilin, epidermin, and ancovenin [20]. Pipecolic acid (piperidine-2-carboxylic acid) accumulates in pipecolic acidemia. It can be associated with some forms of epilepsy [21]. Octopine is the first opine discovered in octopus muscle and later in crown gall tumors. Some opines are conjugates of proteinogenic amino acids and α-keto acids, which are formed in the tumors induced on plants by the bacterium *Agrobacterium tumefaciens* [22]. L-Orn is one of the products of the action of the enzyme arginase on L-Arg, creating urea. Therefore, Orn is a central part of the urea cycle. In bacteria, such as *Escherichia coli*, Orn can be synthesized from L-glutamate [23]. Sarcosine, also known as *N*-methylGly, is formed from dietary intake of choline and from the metabolism of Met, and is rapidly degraded to Gly, which, in addition to its importance as a constituent of protein, plays a significant role in various physiological processes as a prime metabolic source of components of living cells such as glutathione, creatine, purines and Ser [24].

Amino acids having the D-configuration also exist in peptide linkage in nature. The vancomycin related antibiotics bind to the bacterial cell wall D-Ala-D-Ala terminal group, blocking the process of wall-building of Gram-positive bacteria under aerobic and anaerobic conditions [25]. The protective antigen (PA) and the poly-γ-D-glutamic acid (γDPGA) are essential for the virulence of *Bacillus anthracis* (anthrax) [26]. The antibiotic gramicidin-S contains the nonproteinogenic amino acid L-Orn and two residues of Phe in the D-configuration [27]. D-Val occurs in antinomycin-D, a potent inhibitor of RNA synthesis [28].