Preface

Free and Peptide-Bound D-Amino Acids in Chemistry and Life Sciences

'L'universe est un ensemble dissymétrique...'

'The universe is asymmetric and I am persuaded that life, as it is known to us, is a direct result of the asymmetry of the universe or of its indirect consequences. The universe is asymmetric...'

Louis Pasteur, 1874

Knowledge of the formation of D-amino acids (D-AAs) in alkali-treated proteins dates back as early as 1909. The hampered enzymatic digestion and drastically lowered nutritional utilization of the resulting products were already recognized.

Although, in the late 1930s, the presence of poly-γ-D-glutamic acid in the bacterial cell envelope of the virulent *Bacillus anthracis* was realized, and, in the 1940s, a rapidly increasing number of microbial peptide antibiotics containing frequently D-AAs was discovered, their natural occurrence was rather considered as peculiarity of microorganisms.

Notably, γ-(D,L)-polyglutamic acid (γ-PGA) is the major component of the viscous sticky mucilage of *Bacillus subtilis* (natto) that is nowadays widely used for the mass production of traditional fermented soybean foods in Asian countries. Many species and strains of *Bacillus* employed in the biotechnology industry produce γ-PGA with varying ratios of D- and L-enantiomers.

At the beginning of the 1970s, a highly noticed concept for the chemotaxonomy of prokaryotic organisms was developed, and it is still in use. It is based on the sequence and configuration of the AAs forming the cross-linking peptides of the so-called peptidoglycan. Remarkably, D-Ala and D-Glu, as well as several other D-AAs were common in these peptides, and essential for the structure and function of the bacterial cell envelope.

Consequently, the detection of abundant L-AA racemases and isomerases in these microorganisms was not surprising. However, in this context it is worth noting that, until the late 1980s, it had not been fully realized in the discussion on possible harmful effects related to D-AAs that the common consumption of microbially fermented foods and beverages is inevitably connected to the intake of D-AAs. This is of nutritional importance, since an abundance of foodstuffs such as fermented dairy and soybean products, sour dough bread, fermented meat, sausages, and condiments or alcoholic fermented beverages constitute a considerable part of the human diet.

To continue, probiotics, representing health food and feed, are defined as *'live microorganisms which when administered in adequate amounts confer a health benefit*

on the host'. Probiotics are preparations mostly of selected lactic acid bacteria or bifidobacteria which contain D-AAs in their peptidoglycan. Thus, human beings have a steady intake of nutritional D-AAs originating from bacteria. These considerations can be extended to the indigenous bacterial flora of the gastrointestinal tract.

It is also worth mentioning that peptide chemists were always confronted with the need to confirm success of a synthesis also with regard to the optical purity of their end products. Consequently, the challenge to develop methods and reagents to prevent the enantiomerization (commonly termed 'racemization') was and still is a topic in conventional and solid-phase synthesis of peptide drugs and natural products. Establishment of the configuration of AAs in natural and synthetic products required methods for the reliable and sensitive determination of the configuration of the constituting AAs. Related issues are the use of AAs as chiral catalysts, development of methods for the enzymatic, chemical, or physical large-scale separation of mixtures of DL-AAs resulting from industrial syntheses.

In the 1970s and 1980s, concerns about possible toxic effects of D-AAs stimulated much research on the nutritional and physiological relevance of these compounds. With the emerging techniques of capillary gas chromatography (GC) and high-performance liquid chromatography (HPLC), together with the introduction of an arsenal of chiral derivatizing reagents for the indirect separation of AA enantiomers as diastereoisomers, analytical methods became available enabling the separation and quantification of AA enantiomers. In parallel, dedicated chiral stationary phases for the direct enantiomer separation of DL-AAs by either GC or HPLC, and, just recently, capillary electrophoresis and capillary electrochromatography were developed. Coupling with mass-specific detectors and use of selected-ion monitoring techniques or electrospray mass spectrometry are nowadays standard procedures in many laboratories.

Concerns about the possible harmful effects of D-AAs on animals and human beings were modified by the detection of effective D-AA oxidases in liver and kidney, and common renal excretion of a variety of D-AAs with the urine. D-Amino acid oxidase (DAAO) is a FAD-dependent enzyme and catalyzes stereospecifically the oxidative deamination of D-isomers of neutral and polar amino acids. Besides the established oxidative elimination of such D-AAs in mammals, there is evidence that DAAO plays a regulatory role in human and mammalian brains, where it metabolizes the glial-derived neurotransmitter D-Ser, and thus modulates the concentration of D-Ser. D-Ser may be involved in controlling the extent of *N*-methyl-D-aspartate (NMDA) receptor-mediated neurotoxic insults observed in disorders such as schizophrenia. NMDA is a potent neuroexcitatory AA that has specific action as an agonist for one of the glutamate sub-type NMDA receptors in the central nervous system of invertebrates and vertebrates. NMDA Receptors play an important role in learning and memory. To summarize, there is little doubt that certain free and simply conjugated D-AAs occupy physiological niches and have functions not performed by the dominating L-AAs. In marine animals, it has been hypothesized that D-AAs play a role in the regulation of the organisms osmomolarity in response to the changing environment.

Furthermore, an impressive and steadily increasing number of peptides containing D-AAs have been isolated from living organisms. In both invertebrates (terrestrial and marine snails, clams, crayfish, and lobster) and vertebrates (frogs and reptiles), peptides have been discovered that contain in certain positions D-AAs which are

indispensable for the full bioactivities of these compounds. These peptides act as transmitters and neuromodulators, and also as neurohormones. Prominent members are achatins with D-Phe and fulicins with D-Asn from the subesophageal and cerebral ganglia of the African giant snail *Achatina*, and contryphans, containg D-Trp, from the fish-hunting snail *Conus radiatus*. Further, dermorphins and deltorphins, opioid peptides with D-Ala or D-Met, D-Ala, D-Leu, respectively, were isolated from the skin secretions of South American frogs *Phyllomedusa*. And antimicrobial peptides containing D-*allo*-Ile or D-Leu have been found in the skin secretion of species of the European toad *Bombina*. Unravelling the biosynthesis of these peptides showed that specific peptide isomerases converted the L-configuration of certain AAs in the final peptide sequence to D.

Latest spectacular research in this context is the finding that the male of the duck-billed platypus (*Ornithorynchus anatinus*), representing an egg-laying mammalian, synthesizes a defensin-like peptide with D-Ala, and that a L-to-D-peptide isomerase is found in the venom. Moreover, peptide isomerase activity has been detected in platypus tissues. It is intriguing to predict that similar kinds of enzymes might exist in higher mammals and play previously unanticipated biological roles. One might even speculate about a 'shadow world' of peptides containing D-AAs in living organisms, including man, which escape the usual fast metabolic turnover, and which are still waiting to be discovered and explored. However, despite the fact that the occurrence of D-AAs in several invertebrates and mammalia were already recognized in the mid-1960s, free, and peptide- or protein-bound D-AAs were commonly named 'unnatural' or 'unphysiological' AAs, a terminology that can still be found in some textbooks.

Among various nonenzymatic post-translational modifications of proteins, racemization and isomerization of amino acids in proteins, and the resultant proteins containing D-amino acids are especially important in the development of various age-related diseases such as eye lens cataract *etc*. In the membrane proteins of aging erythrocytes, D-Asp and D-iso-Asp residues were detected, and the mechanism *via* succinimide formation for the nonenzymatic isomerization and racemization of the L-Asp was unravelled. This conversion would lead to time-dependent, drastic structural changes. D-β-Asp-containing proteins were detected in age-related cataract, pinguecula, age-related macular degeneration, and hereditary (corneal dystrophies) diseases. A similar mechanism is also regarded to be responsible for the nonenzymatic conversion of L-Asp to D-Asp in certain positions of the amyloid protein involved in *Alzheimer*'s disease. Similar structural alterations are to be expected for all long-living proteins and should be targets for future research. Until recently, the process was considered to be irreproducible, leading inevitably to structural damage of proteins, resulting in severe cell damage and, finally, disease. No therapy for such nonenzymatic secondary-structural alterations of proteins was in sight. However, there is solid evidence that a ubiquitous protein-repair enzyme, the protein L-isoaspartyl (D-aspartyl) *O*-methyltransferase (PIMT), is capable to reverse this reaction. This finding might be also the starting point for therapy. To continue, the question arises how stereospecific at all the ribosomal biosynthesis of proteins is. Is it one-hundred percent, or is a small fraction of D-AAs included? This issue requires not only sensitive and extremely reliable methods for the detection of small amounts of D-AAs in the proteins, but also

rigorous design and professional performance of the experiments in order to exclude contaminations and artefacts.

Beyond the tasks related to biological and nutritional sciences as outlined above, the fundamental question on the origin and dominance of L-AAs in life, as we know it, is still not satisfactorily answered. Abiotic synthesis of AAs on primordial Earth or under extraterrestrial conditions should provide truly racemic (DL) mixtures of AAs. The genesis of the observed enantiomeric excesses of certain non-proteinogenic AAs such as L-isovaline in meteorites is still a matter of debate. Experiments simulating interstellar processes and conditions have started and might reveal that the L-preference of AAs is an inherent feature of the universe. To answer this question (among others), in 2004 the *Rosetta Mission* spacecraft was launched and is on the way to the comet 'Chury' (*67P/Churyumov–Gerasimenko*). Hopefully, it will release in 2014 the robotical Lander Philae. That is equipped with a sophisticated GC/MS module capable of resolving and identifying also AA enantiomers with the aid of capillary columns coated with chiral stationary phases.

The emerging relevance of D-AAs, be it in the free, or peptide- or protein-bound states, and the need for a forum to exchange ideas and hypotheses concerning the role of D-AAs led to the foundation of the '*D-Amino Acid Research Society*' in Japan a few years ago and resulted in the '*First International Conference on D-Amino Acid Research*' held in Awaji City, Japan, in July 2009. Consequently, the majority of contributions published in this volume result from this conference and reflect also the long-standing interest of the Editors, and their co-workers and collaborators in the field of D-AA research.

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