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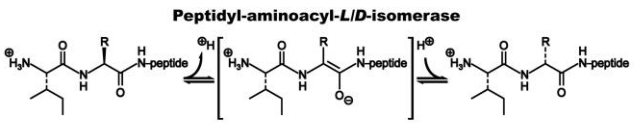
FWF P22782: **A search for vertebrate peptides containing D-amino acids**

As a subtle posttranslational modification, some natural peptides contain a D-amino acid with remarkable effect on their properties and biological function. We investigate the Chemistry of the conversion reaction and the resulting change in physico-chemical properties of the peptides.

In multicellular organisms many vital functions are mediated by peptides. Some of these peptides contain a D-amino acid in a well-defined position. To date, in all vertebrates and several invertebrates the D-amino acid is the second residue of the mature peptide whereas it has been found in other positions in invertebrates. Vertebrate peptides comprise dermorphins, deltorphins, and the peptide antibiotics bombinin H from frog skin, as well as a C-type natriuretic peptide (CNP) and beta-defensin-like peptide (DLP) from the venom of male *Platypus*, a primitive mammal. In case of the opioid peptides, the all L-forms are inactive, whereas subtle differences in activity and physicochemical properties between the two diastereomers of bombinin H have been observed. From skin secretions of *Bombinae*, an enzyme has been isolated which catalyses the posttranslational inversion of the stereochemistry of the respective L-amino acids present in the precursor of bombinin H. This L/D-isomerase acts exclusively on the second residue. Genes encoding polypeptides related to the frog skin enzyme are present in many vertebrates including man supporting the idea of the existence of diastereomeric peptides in higher mammals. A detailed study of the substrate specificity of the frog isomerase and subsequent data base search has led to the identification of potential substrates, which include hormones, antibacterial peptides etc., for which a D-form could exist as well. Based on this knowledge, we mainly focus on the questions, whether i) the diastereomeric form of a few selected peptides can be detected in animal tissues and thus the presence of the D-residue be directly confirmed, and ii) the biological activity and/or physicochemical properties of the peptides are modulated by the stereoinversion. The D-amino acid could crucially affect receptor interactions, folding propensities or polypeptide



turnover in these peptides and thus have far-ranging consequences on their biological function.

In particular, we have studied the effect of a predicted stereochemical modification in position 2 of peptide aDrs from the skin of the Mexican tree frog (*Pachymedusa dacnicolor*), which may represent an example of an amyloid structure involved in innate immunity, albeit one where the amyloid, assembled form is a deposit form rather than the active form (Gößler-Schöfberger et al., 2009). The investigation of aDrs aggregation has revealed interesting aspects of superstructural organization and polymorphism (Gößler-Schöfberger et al., 2012). In fact, this modification results in a fundamental change in superstructural organization, which is particularly remarkable considering that the site of stereoinversion is not localized within the aggregation-prone sequence and, furthermore, this modification can be enzymatically introduced. Therefore, D-amino acids could play a role in natural strategies to control protein aggregation in cases where amyloid with its unique properties is a functional, integrated component of the organism, and, moreover, also in the pathogenesis of protein deposition disorders, i.e., certain neurodegenerative diseases or amyloidoses, where peptide turn-over, cross-seeding effects, and a differential toxicity of superstructure morphologies are important factors.

Keywords: peptide biosynthesis, chirality, D-amino acid, opioid peptide, isomerase, peptide antibiotic