Figuring Out the Configuration

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Imagine being able to reach into your sample vial and fish out a single peptide molecule. Perhaps the molecules would be full of thermal energy and difficult to catch, but you might just be able to grab one before it wriggled away. Holding the N-terminus in one hand and the C-terminus with the other, imagine stretching the molecule into an extended conformation (Fig. 1). Looking down the backbone, from the N-terminus to the C-terminus, with the peptide bonds oriented vertically, you would see side-chains sticking out to the sides: some to the left, others to the right. If the first side chain were sticking out to the right (roughly "east-northeast"), then the second would stick out the left ("west-southwest"), and this alternating pattern would repeat along the length of the molecule. This description applies to nearly all natural peptides and is a consequence of the amino acid building blocks' L stereochemistry (technically the backbone carbonyl C→O of the first amino acid would have to be pointing downwards, or "south", for the description to be accurate for an all-L peptide). Occasionally, however, depending where your sample came from, you might come across a molecule with an unusual structure; one in which two or three consecutive side chains were sticking out on the same side (on the right, for example: an "east-northeast" followed by an "east-southeast"). Such a pattern would be explained by the presence of a D-amino acid, flanked by L neighbours. D-Amino acids are rare in nature, but when they do occur they can have effects on the structure and function of peptides (Kreil, 1997). For peptide chemists, the appearance of these alternative configurations as synthetic by-products is simply a nuisance that is to be avoided (Anderson and Callahan, 1958). This article highlights some recent literature on the detection of D-amino acid residues and of other types of stereoisomerism in peptides.

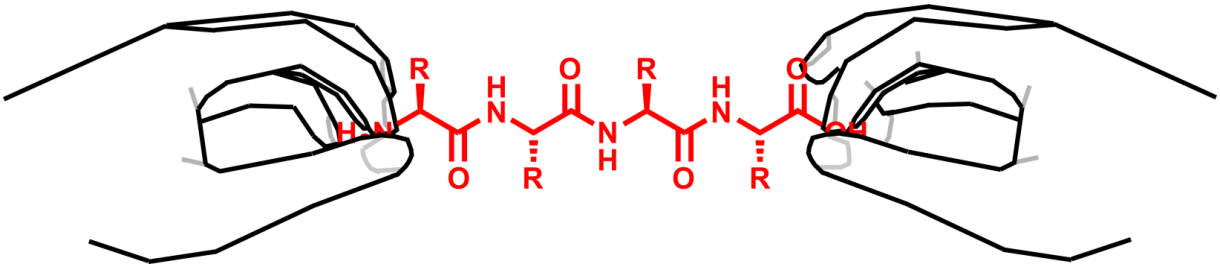

Fig. 1. Stretching the peptide backbone into an extended conformation.

ENIGMATIC ISOMER

The occurrence of D-amino acids might explain a finding phenomenon that has been puzzling insect physiologists for over two decades – the intriguing isomerism of a peptide hormone found in cicadas. Writing in *Analytical and Bioanalytical Chemistry*, König *et al*. describe work towards uncovering the structural basis of the isomerism (König *et al*., 2017). The peptide in question is secreted by glands attached to the cicada's brain, and its function is to stimulate the breakdown of glycogen elsewhere in the insect's body. Edman sequencing and mass spectrometry (MS) had previously indicated that the peptide's sequence was pGlu-VNFSPSWGN-amide and, accordingly, it co-eluted in HPLC with the corresponding synthetic all-L peptide (pGlu = pyroglutamic acid; Veenstra and Hagedorn, 1995). However, HPLC analyses also revealed the presence of a second peptide with exactly the same sequence. This unknown variant had a shorter retention time but was otherwise indistinguishable from its validated counterpart. Now, König *et al*. report the use of ion mobility spectrometry (IMS) to elucidate the unique characteristic(s) of the unknown peptide.

As a method of separating gas-phase ions on the basis of shape (average cross-sectional area), IMS is particularly good at analysing isomers. The influence of shape, rather than mass *per se*, permits analyses that would be fundamentally impossible using the related technique of MS. With modern instrumentation though, it is possible to perform MS as well: it can be done before IMS (e.g., using a quadrupole to pre-select ions) and then again afterwards (e.g., time-of-flight MS to identify the ions as they "elute"). To characterise the unknown peptide, König *et al*. added another layer of complexity by first fragmenting the ions, and then using IMS to analyse the fragments. When peptides extracted from the cicada glands were analysed in this way, the authors observed divergent behaviour for some of the fragments. These parts of the sequence, they reasoned, must be where the structural differences lie. On this basis, the authors speculate that a portion of the sequence (Pro-Ser-Trp) might contain one or more D-amino acids, or alternatively that *cis*/*trans* isomerisation of the Ser-Pro peptide bond might be responsible. It will be interesting to learn further details as this work progresses.

CHIRAL AMINO ACID ANALYSIS IN STEREO

Writing in *Analytical Chemistry*, Wang *et al*. describe a method specifically designed to detect D-amino acid residues in peptides (Wang *et al*., 2017). The method is based on derivatisation of the peptides' constituent amino acids, which are obtained *via* acid hydrolysis of the parent material. Treatment of the hydrosylate with a pair of chiral salicylaldehyde esters (the stereogenic center being in an esterified proline moiety) generates distinctive products whose structures depend on the stereochemistry of the amino acid substrates (Fig. 2). The mechanism of the reaction involves condensation of the amino acid's α-amino group with the reagent's aldehyde group, producing an imine that contains at least two stereogenic centres. This process of derivatising one chiral molecule (the analyte) with another (the reagent) is an established approach to chiral amino acid analysis (e.g., use of Marfey's reagent; Bhushan and Brückner, 2004). What is novel about Wang and colleagues' method is that it uses *two* reagents: one "light" (normal benzoyl group); the other "heavy" (deuterated benzoyl group). One of four different imine products is formed, but the stereospecificity of the reaction means that only two of them really matter. For example, (*S*)-phenylalanine reacted preferentially with the (*S*) form of the reagent to form the "light" (*S*,*S*) product, whilst (*R*)-phenylalanine reacted with the (*R*) form of the reagent to form the "heavy" (*R*,*R*) product (Fig. 2). The differentially-tagged enantiomers can be identified using MS without the need for standards. The authors performed experiments to confirm that the reactions were indeed under kinetic control, and they made measurements of "chiral recognition ability" for nineteen pairs of enantiomers (proline did not react). They then used their method to analyse hydrosylates of peptides (3-mers, 5-mers and a 7-mer) containing single D-amino acids. Further validation may be needed before the method can be used for routine analyses of peptides, but it nevertheless appears to be a promising solution for what is a challenging problem in amino acid analysis.

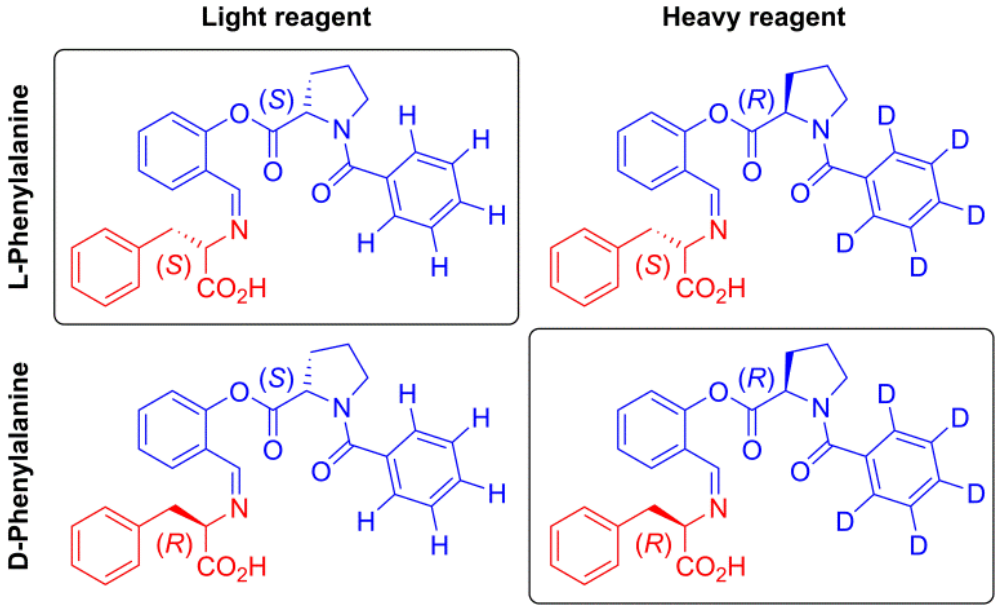

Fig. 2. The enantiomers of phenylalanine undergo different stereospecific reactions when treated with a mixture of chiral derivatisation reagents. Structures enclosed in boxes are the major products observed by Wang *et al*. Imines are all drawn as their *cis* isomers for the sake of clarity.

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