Proteases break the mirror of chirality

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Proteases are widely assumed to enforce strict stereochemical selectivity, limiting activity to L-peptide substrates. Li *et al.* overturn this paradigm, revealing that some natural proteases can act "ambidextrously", efficiently cleaving both L- and D-peptides and redefining assumptions about enzyme specificity and peptide stability.

Biology is built on asymmetry: proteins are composed almost exclusively of L-amino acids. This molecular handedness underlies the specificity of enzyme–substrate recognition and has long been assumed to impose strict stereochemical constraints on proteolysis. Yet Li *et al.* now show that this rule is not absolute, revealing that some natural proteases can efficiently cleave both L- and D-peptide substrates[1]. By adapting synthetic combinatorial substrate profiling technology for proteases[2], they extend its scope in a striking way: constructing libraries composed entirely of D-amino acids. This innovation enables a systematic interrogation of whether natural proteases can recognize and cleave mirror-image substrates. The answer, unexpectedly, is yes.

The term "ambidextrous", which originally describes individuals capable of using both hands with equal precision, whether slinging stones or drawing a bow, finds a molecular analogue here. Li *et al.* reveal proteases that can strike both enantiomeric targets with comparable efficiency. Specifically, papain, cathepsin B, and mouse carboxylesterase 1c cleave both L- and D-peptides of identical sequence with substantial catalytic efficiency.

This finding stands in contrast to decades of work emphasizing strict stereochemical fidelity. Inverting the chirality of a substrate typically abolishes enzymatic activity, whereas simultaneous inversion of both enzyme and substrate restores function[3]. A few exceptions have been reported, including proteases that tolerate isolated D-amino acids and bacterial enzymes that process D-peptides in the context of antibiotic resistance[4]. Fully D-peptides are widely assumed to be proteolytically stable and are frequently incorporated into therapeutics to enhance serum persistence[5,6].

Li *et al.* overturn this assumption through a systematic and quantitative approach. Using combinatorial D-peptide libraries, they identify sequence contexts in which proteases not only tolerate D-amino acids but efficiently cleave fully D-configured substrates. Crucially, when both enantiomers of optimized substrates are synthesized, the same native L-proteases process both forms. This is not nonspecific degradation but sequence-dependent, enzyme-mediated catalysis.

What distinguishes these ambidextrous proteases from their more selective counterparts? A key insight is that they tend to exhibit broader substrate specificity and more permissive binding pockets. Structural and computational analyses suggest that L- and D-peptides bind in distinct orientations within the same active site, with catalysis preserved through alternative interaction networks.

The implications extend beyond fundamental enzymology. The authors demonstrate a practical application by engineering a fully D-peptide cleavable linker for antibody–drug conjugates (ADCs), leveraging cathepsin B activity. This linker exhibits improved plasma stability, enhanced hydrophilicity, and efficient cleavage under lysosomal conditions, yielding potent antitumor activity. Since cathepsin B is already a widely used prodrug activation target[7], extending the toolkit with a serum-stable option for this enzyme will be useful for drug delivery in the future. Furthermore, the D-amino acid binding pockets could be explored using unnatural amino acids as has been done for L-amino acids[8].

More broadly, these findings challenge the widespread

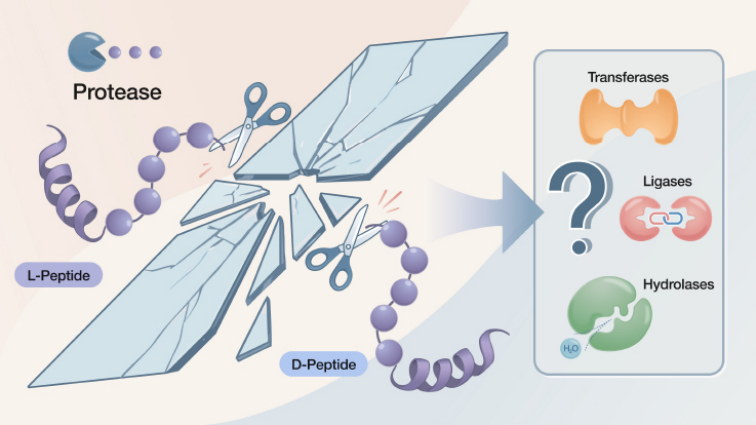

Fig. 1 Ambidextrous protease specificity could extend to other enzymes. A schematic of a protease (blue scissors) that can cleave both the L-and D-versions of a purple peptide substrate that has been reflected across a mirror plane. The arrow points to the possibility of the same behavior for other peptide-interacting enzyme classes.

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assumption that D-amino acids confer universal proteolytic resistance. Instead, stability emerges as a context-dependent property, shaped by both sequence and the proteolytic environment.

There are over 400 distinct types of post-translational modifying (PTM) enzymes, broadly categorized as transferases, hydrolases, and ligases, that collectively regulate protein functions through the addition, removal, or cleavage of chemical groups. Many of these enzymes recognize their substrates as extended peptide conformations which often resemble β-strands within target proteins, enabling sequence-dependent modification across diverse biological contexts[9]. In this light, it is intriguing to consider whether ambidextrous recognition might extend beyond proteases, raising the possibility that other PTM enzymes could also accommodate D-amino acid-containing substrates (Fig. 1).

Perhaps most importantly, it serves as a reminder that even the most entrenched assumptions in biochemistry can yield to careful experimentation, and that the mirror image of a molecule is not always invisible to biology.

COMPETING INTERESTS

T.C.D. and C.S.C. declare no competing interests.

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ADDITIONAL INFORMATION

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