Designing peptides to meet the challenge of the next generation of druggable targets

Review Article

Targeting protein–protein interactions (PPIs) is a new challenge in expanding druggable space. Cyclic peptides show promise in targeting PPIs and to better understand their structure–activity relationships a research team at Tufts University, USA, has analyzed a series of designed, well-structured cyclic hexapeptides and use simulations and experimental techniques to understand their global structural ensembles. They discovered a previously unappreciated role for β-branched residues in stabilizing specific conformations of cyclic hexapeptides, and their approach shows promise in the prediction of structure–activity relationships for drug development. Their work was rewarded by being on the front cover of Biophysical Journal, Volume 116, Issue 3.

The value of cyclic peptides in targeting PPIs

Cell homeostasis depends on a fine-tuned network of an estimated 130,000 and 600,000 protein–protein interactions (PPIs) in the human interactome, which makes PPIs promising therapeutic targets (see 1, 2). While small molecules are unable to target the large and relatively featureless flat surfaces involved in PPIs, specifically designed peptides can meet this challenge. With a long history of use in therapeutics, peptides are regarded as safe and well tolerated, and peptides that interfere with PPIs have been shown to modify several cellular processes, making them promising therapeutic tools. One class of peptides that is particularly interesting is cyclic peptides that are pre-organized for binding and offer additional advantages, including reduced proteolytic degradation, increased bioavailability, membrane permeability, and half-life in plasma, and improved oral uptake.

Fine-tuning cyclic peptide conformation

The ability of cyclic peptides to bind to surfaces involved in PPIs is conformation-dependent. Prof. Yu-Shan Lin's research group, in the Department of Chemistry at Tufts University, had already shown that for small cyclic hexapeptides, two dihedral angles need to change coherently to enable conformational switches. These could be either the φ and ψ angles of the same residue (φ_i and ψ_i) or the ψ angle of one residue and the φ angle of the next residue (ψ_i and φ_{i+1};3).

Following up on this, they studied the sequence-structure relationships of conformations of cyclic peptides. Residues in a hexapeptide cyclo-(GGGGGG) were gradually substituted with the branched amino acid valine and then computationally analyzed to find cyclic peptides predicted to have a single highly populated conformation in aqueous solution (4). They discovered one peptide, cyclo-(VVGGVG), or P7, that was unique with 80% of the population forming two type II β turns at residues 2–3 and 5–6 in the simulation. Their latest investigation (5) found that β-branched residues at position 1 of P7 played an important role in stabilizing this specific conformation. Pairing these computational insights with experimental work with collaborators Ashleigh Cummings and Prof. Joshua Kritzer, they started by experimentally verifying the structure predictions for P7, and also a less well-structured control, cyclo-(VVGVGG), or P6, using nuclear magnetic resonance (NMR) spectroscopy of synthetic peptides, along with a number of P7 analogs.

Peptide synthesis and cyclization

The linear peptide with a deprotected N-terminus was synthesized at 0.05 mmol scale on Wang resin preloaded with Fmoc-glycine using a Tribute® automated peptide synthesizer with ultraviolet deprotection monitoring and infrared heating on all couplings. The peptide was cleaved from the resin using 4% H2O (v/v) in a solution of trifluoroacetic acid for 3 h at room temperature, filtered, dried and dissolved in 50% H2O: 50% acetonitrile before being lyophilized. Prof. Yu-Shan Lin was pleased with the performance of the peptide synthesizer, commenting that, *"Tribute produced linear hexapeptides with excellent yield and high purity, allowing us to use crude peptide in subsequent solution cyclization reactions".* The lyophilized crude peptide was directly used for cyclization at room temperature for 1 h with HATU and DIPEA in DMF, before quenching with trifluoroacetic acid. Peptides were purified by high-performance liquid chromatography to give >95% purity.

Table 1: Sequences of P7, P7 analogs, and the P6 control

Peptide	Sequence
P7	VVGGVG
V1A	**A**VGGVG
V2A	V**A**GGVG
V5A	VVGG**A**G
V1I	**I**VGGVG
V1L	**L**VGGVG
V1T	**T**VGGVG
V1S	**S**VGGVG
P6	VVGVGG

NMR confirms predictions

While P7 and P6 have the same residue composition there are large structural differences between these two cyclic peptides. The wide span of its amide chemical shifts suggests that P7 is well structured, while the Nuclear Overhauser Effects (NOE) data indicate that this cyclic peptide contains two types II or II′ β-turn structures at positions 5–6 and 2–3, matching the simulation prediction. P6, on the other hand, has β-turns at various positions. Studies with variable-temperature NMR indicated that the solution ensemble of P6 has several interconverting, internally hydrogen-bonded structures while the P7 ensemble is dominated by a single structure.

Simulations involving replacing each of the three valines in P7 with alanine showed that β-branching at position 1 is critical to the stability of the overall $\beta_{II}+\beta_{II}$ structure. To confirm this, two P7 analogs, V1I and V1L, were analyzed experimentally using NMR. The results showed that V1L populates an ensemble with varied β turns and no predominant β turn location, whereas V1I has a predominant structure that is very similar to P7 but with a somewhat lower degree of structure. The effect of β-branching was also analyzed using the P7 analogs V1T and V1S, which were both more well structured than V1L but not as well structured as P7 or V1I.

The peptides could be ranked in order of decreasing level of structure based on the variable-temperature data: P7 > V1I > V1T > V1S > P6 > V1L. This order matched chemical shift data and NOE data, and was consistent with predictions from the simulations based on the percentage population of the most populated cluster for each

peptide. The NMR data therefore confirmed that the simulations identified the dominant structure for each peptide in solution and also predicted the overall population of that structure compared to similar peptides. A thermodynamics analysis of the simulation results also provided molecular-level insight into the findings.

A promising approach for designing well-structured cyclic peptides

Small changes in the sequence of a cyclic peptide can lead to large changes in the solution ensemble, which mirrors the difficulty in predicting structure–activity relationships (SAR) for macrocyclic compounds. This research team at Tufts University, however, has succeeded in developing simulations and validating them with experimental data. This demonstrates how efficient molecular dynamics methods can be used to systematically characterize cyclic peptide structural ensembles and identify well-structured sequences. Their approach promises to help drive the development of fully predictive computational SAR models that will be invaluable in the development of drugs based on cyclic peptides and other macrocyclic molecules.

References and further reading

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