BIOLOGICS

Break it up

Aggregation into intractable deposits is a problem that plagues many potential peptide and protein drugs, but a new and general rational design approach could help address this, according to recent research published in the *Proceedings of the National Academy of Sciences*. Zurdo and colleagues show that by altering selected amino acids predicted to be important for aggregation of the peptide hormone calcitonin, variants with significantly reduced aggregation propensity can be produced without any loss of biological activity.

Peptides and proteins have become increasingly popular as drug candidates in recent years, but despite considerable therapeutic potential many are rendered ineffective because of their propensity to aggregate irreversibly. Aggregation can not only compromise bioavailability and therapeutic activity, but might also lead to undesirable immune responses to the drug.

However, recent research in the field of protein-deposition diseases has shown that a method that uses a combination of simple physicochemical parameters can be used to quantitatively predict the effect of amino-acid changes on the aggregation behaviour of polypeptides associated with such diseases. So, the authors set out to investigate if a similar method could be used to design variants of a therapeutic peptide with a reduced propensity to aggregate.

The peptide chosen, human calcitonin (hCT), is a 32-residue hormone involved in calcium regulation that has been used to treat conditions such as osteoporosis. However, for several years salmon calcitonin (sCT) has been used clinically in preference to hCT, despite side effects related to its non-human nature, because of its reduced tendency to aggregate.

Using information from studies of peptide fragments of hCT focused on those residues thought to have a key role in the activity and aggregation of the full peptide, Zurdo *et al.* designed a series of full-length variant CT peptides. Modifications were made following a number of principles. In particular, changes were made with the aim of disrupting hydrophobic patches in highly aggregation-prone regions of hCT, and stabilizing interactions within hCT that could help prevent aggregation. Changes to residues thought to be important for biological activity were avoided, and the maximum number of changes was also limited to six to reduce the likelihood of losing activity or causing side effects.

Computational prediction of the aggregation propensity of several hundred sequences was used to select three full-length hCT variant peptides for experimental testing. A significant reduction in the rate of aggregation of all three re-engineered variants was found compared with hCT, and two were also better than sCT in this respect. Most importantly, these two variants showed increased physiological activity compared with hCT both *in vitro* and *in vivo*.

Such hCT variants could provide an improvement over sCT for the treatment of those conditions for which this peptide is currently used. More generally, the method developed by the authors could have a significant impact on the development of many polypeptide-based drugs, potentially allowing optimization of their production, formulation, potency and shelf-life.

Peter Kirkpatrick

References and links

ORIGINAL RESEARCH PAPER Fowler, S. B. *et al*. Rational design of aggregation-resistant bioactive peptides: reengineering human calcitonin. *Proc. Natl Acad. Sci. USA* **102**, 10105–10110 (2005)
FURTHER READING Chiti, F. *et al*. Rationalization of the effects of mutations on peptide and protein aggregation rates. *Nature* **424**, 805–808 (2003)