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Rational design of peptide drugs: avoiding aggregation

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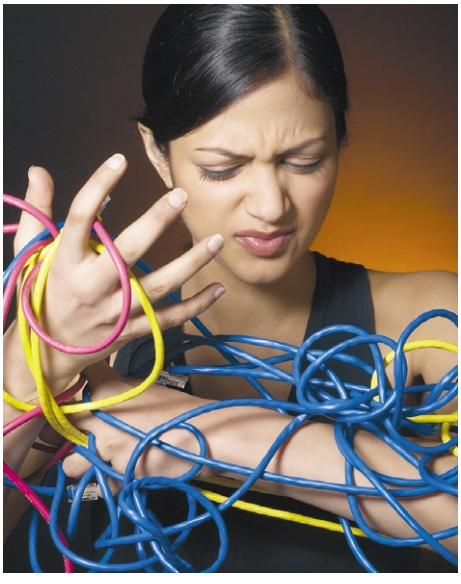
Peptides and proteins hold great promise for the treatment of human diseases but many hopeful leads have been abandoned because of aggregation problems encountered during production or storage. Now, in a proof-of-principle experiment, Jesús Zurdo (Research Director and co-founder of biotechnology spin-out company Zyentia, Cambridge, UK), Chris Dobson (Professor of Chemistry at the University of Cambridge and Zyentia co-founder) and colleagues have used a predictive algorithm to design a bioactive variant of human calcitonin with a reduced propensity to aggregate [1]. In the future, claims Zurdo, algorithms of this type should allow researchers and drug companies to reduce troublesome aggregation of many potentially useful peptides.



Aggregation: a sticky problem

Most of the proteins that our cells produce are soluble in their normal surroundings. However, try to overexpress those same proteins in bacteria or to produce concentrated solutions of bioactive peptides for human therapy and the result is often an inactive, unstable, potentially toxic aggregate. Although acylation or pegylation of proteins or the use of liposomes for drug delivery can sometimes circumvent this problem, Zurdo and his colleagues are taking another approach towards reducing the aggregation of potentially therapeutic peptides and proteins.

'Our groups have been working for many years on protein misfolding and aggregation and its relationship with human disease', explains Zurdo, 'trying to understand the mechanisms by which a protein forms amyloid fibrils or toxic aggregates'. These studies have resulted in the development of algorithms based on physicochemical parameters that can predict how amino acid substitutions will affect the aggregation behaviour of peptides.

Testing aggregation algorithms

To investigate the potential for their algorithms in drug discovery, the researchers turned to human calcitonin. Salmon calcitonin is sometimes used to treat osteoporosis; human calcitonin, however, has a limited pharmaceutical potential because of its marked tendency to aggregate. 'We evaluated more than 600 variant human calcitonin sequences *in silico* on the basis of their aggregation propensity as predicted by our algorithms', says Zurdo, 'and then chose three variants for testing experimentally'. The behaviour of the variants complied with the researchers' predictions in that they were stable in solution and failed to aggregate [1]. Importantly, they also stimulated the calcitonin receptor more effectively than non-variant human calcitonin.

'Right now, there is a whole list of agents that affect bone formation, including salmon calcitonin', comments Stuart Silverman (Clinical Professor of Medicine and Rheumatology at the University of California Los Angeles School of Medicine, USA), 'so these results are not a breakthrough for the treatment of osteoporosis. However, for medicine in general, increasing the bioavailability of peptides is important, so as a proof of process, this research is fascinating.'

> **'…for medicine in general, increasing the bioavailability of peptides is important…'**

Joost Schymkowitz (joint group leader of the SWITCH Laboratory at the Vrije Universiteit Brussel, Belgium), who is one of the developers of an alternative algorithm called TANGO, also used to predict protein aggregation [2], comments that, 'it would be a major breakthrough in drug discovery if we could find ways to redesign potentially therapeutic peptides so that they no longer aggregate but remain active. This new research proves that you can do this for calcitonin but this may not be true for all peptides. The challenge now is to refine our algorithms so that aggregation of peptides

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and proteins can be reliably reduced without loss of biological activity or changes to the stability of their native structures.'

Future applications

Zyentia has no plans to take human calcitonin variants into clinical development. Instead, the company has successfully applied its technology to other biopharmaceuticals that are hardly used because of aggregation problems, for example, glucagon. 'This peptide is sometimes used by diabetics to treat hypoglycemic shock but it is extremely insoluble and difficult to handle', notes Zurdo. Zyentia also seeks to license its technology to other pharmaceutical and biotechnology companies.

Finally, Zurdo and Schymkowitz are using their algorithms to search for inhibitors of the pathogenic aggregation that underlies Alzheimer's, Parkinson's and other neurodegenerative diseases [3]. 'By understanding which regions of amyloid-forming proteins drive their aggregation, we have been able to generate compounds that halt the pathogenic process', says Zurdo, adding that these are now in pre-clinical development at Zyentia.

References

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2 Fernandez-Escamilla, A.M. *et al.* (2004) Prediction of sequence-dependent and mutational effects on the aggregation of peptides and proteins. *Nat. Biotech.* 22, 1302–1306

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Endocannabinoids mediate age-old pain suppression

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Stress-induced analgesia (SIA) is a phenomenon where the affected individual does not feel the effects of a physically harmful experience. Although long-documented, it was not until the 1970s that researchers discovered that about half the effects of SIA are mediated by endogenous opioids [1]. 'The question is', says Daniele Piomelli (http://www.ucihs.uci.edu/pharmaco/people/faculty/piomelli.html) from the University of California, Irvine, USA, 'what about the rest?' Now he and Andrea Hohmann (http://www.uga.edu/psychology/faculty/ahohmann.html) from the University of Georgia, USA, have shown that endogenous cannabinoids also contribute to SIA [2].

'endocannabinoids are produced in brain areas involved in emotions'

Piomelli describes the most famous episode of SIA, experienced by the British explorer David Livingstone as a lion attacked him on an expedition in Africa. 'The lion grabbed him and as he was being bounced up and down, he felt nothing, he was very calm. The body shuts off pain signals, because it makes more sense to focus your attention and resources toward escaping the danger.'

Targets for drug development

The brain produces two endogenous cannabinoids: anandamide and 2-arachidonylglycerol (2-AG); both affect pain. Hohmann targeted a known relay station for SIA, the periaqueductal grey matter (PAG). When she injected antagonists to the cannabinoid receptor CB1 into the PAG of rats, SIA was blocked. Then the team looked directly at endocannabinoid production in the PAG, finding anandamide and 2-AG present.

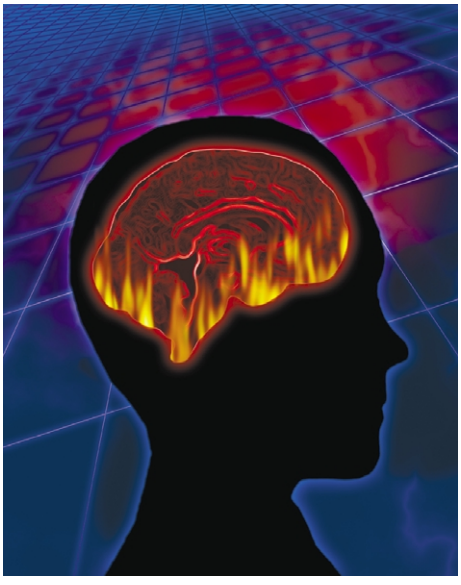
They wanted to show that endocannabinoids were directly mediating SIA, 'could we boost the system up?' asked Piomelli. Each of the endogenous compounds is deactivated by a hydrolyzing enzyme: anandamide by fatty-acid amide hydrolase (FAAH) and 2-AG by monoacylglycerol lipase (MGL). If these deactivators can be shut down, the analgesic effects of the endocannabinoids might increase.

Piomelli previously developed an inhibitor to FAAH that has turned out to have anti-anxiety properties [3]. They used this molecule as a scaffold to construct a specific inhibitor of MGL. Because the inhibitor is not potent enough to work systemically, they injected the compound directly into the brain. Rats whose tails were placed on a hotplate, lingered longer when injected with the inhibitors – evidence of increased analgesia.

Raphael Mechoulam (http://paincenter.huji.ac.il/mechoulam.htm) from the Hebrew University of Jerusalem (not involved in the current work) is hopeful about future uses of MGL inhibitors. 'I can certainly make use of them in neuroprotection', he said. 'They may possibly be used in various neurological states where 2-AG is important'. Hohmann too predicts potential broad-spectrum use of the new inhibitors. 'MGL inhibitors may be useful therapeutically not just for pain but for post-traumatic stress and anxiety-related disorders. We now have a tool to address these possible clinical applications'.

Two endocannabinoids: individual roles

The finding that these endocannabinoids were released in the PAG seemed at first redundant. 'Nature doesn't do things this way, with such a blatant overlap', said Piomelli. He wondered whether they were serving different functions. Intriguingly, the two endocannabinoids were temporally segregated. Whereas, the concentration of 2-AG increased immediately with stress and decreased within a few minutes, 'it was a completely different situation with anandamide, it took seven minutes to go up'. The compounds differed spatially as well. 'When we look for the compounds released in the brain, we see a lot of 2-AG closely associated



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