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Enzyme clicks together its own inhibitor

Kathryn Senior, freelance writer

The enzyme acetylcholinesterase (AChE) has been used to select the chemical building blocks to synthesize its own novel inhibitor. Researchers at the Scripps Research Institute (La Jolla, CA, USA), led by Nobel laureate K. Barry Sharpless, have exploited 'click chemistry' to combine azides and acetylenes to give a 1,2,3-triazole compound that fits perfectly into the active site of AChE. The new inhibitor has activity in the femtomolar range (approximately one-trillionth of a gram per litre), making it several hundred times more potent than currently known AChE inhibitors used to treat Alzheimer's disease (AD)-associated dementia.

Click chemistry

'The idea that enzymes can direct the assembly of good inhibitors is not new, but it is revolutionary in the sense that it has yet to reach its full potential in practical applications,' says M.G. Finn, co-author of the study. Sharpless, Finn and colleagues selected several potential building blocks that could combine to form 98 potential site-specific inhibitors for AChE. The building blocks, based on tacrine and phenanthridinium motifs, were decorated with alkyl azides and alkyl acetylenes of varying chain lengths. Each of the possible combinations was incubated in the presence of AChE at room temperature.

MS analysis of the reactions that occurred showed that only one combination had resulted in the formation of a triazole compound. Control experiments demonstrated that blocking the active site of AChE inhibited the formation of this triazole, indicating that the enzyme's binding cleft was acting as a template for the 1,3-dipolar cycloaddition reaction that resulted in the novel inhibitor

[1]. 'The cycloaddition of azides with alkynes is an ideal reaction for click chemistry,' explains Finn, 'because the two reactive functional groups are only reactive with each other and not with the protein.' Finn emphasizes that the Scripps group did not discover the reaction, but they have exploited its potential in a new way.

The use of click chemistry has important advantages in the discovery and process aspects of drug discovery. 'As we illustrated, the *in situ* approach provides a powerful way to identify promising structures. Allowing the target to make its own inhibitor will often be more efficient than making and testing each of the candidates individually,' explains Finn. He also points out that if one is often faced with the problem of improving a complex matrix of properties including binding affinity, bioavailability and details of formulation, click chemistry makes it easier to synthesize and test large numbers of analogues for this purpose.

'This concept is fascinating,' comments Samuel J. Danishefsky, Kettering Chair and Director of the Laboratory for Bioorganic Chemistry (Sloan-Kettering Institute, New York, USA). 'Sharpless uses relatively straightforward chemistry to present combinatorially a range of acetylenes to react with a range of azides. Ordinarily, such a set-up would give rise to a diverse mixture of products, but because the Scripps team conducted such a reaction in the context of a protein, each core component of the reaction was selected to bind to a different site in the protein,' observes Danishefsky. By bringing the components for the cycloaddition together, the protein selects the optimal spacing between each functional group. 'Since the reaction is likely to be irreversible, it seems that the three-dimensional structure of the protein is being used to control the players in the cycloaddition through totally kinetic means,' he says.

A one-off?

What is not yet clear is if the Scripps group were simply lucky in the reaction they chose to investigate in this study. The click reaction chosen is slow on its own, so any 'hits' that occurred (i.e. any 'clicking together' of the candidate pieces) were likely to result from the pieces being held together in just the right way. 'This suggests that the binding of the components to the target would be just right in this case, otherwise the required connection would not be formed,' says Finn, 'Still, none of us expected the resulting molecule to be as potent as it is, so we might also have stumbled on additional factors that make the binding so tight.' For example, the group looked on the azide and alkyne as 'benign' groups, with the job of only making a connection when their attached binding groups got into the proper position on the enzyme. However, Finn explained that the resulting triazole unit itself might have an added affinity for the enzyme, which would make the resulting inhibitor even stronger than one would be expected based on the binding constants of the two component pieces.

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Danishefsky thinks that the reason the inhibitors are so potent is because they were assembled in a fashion where they had to be potent. 'That is what makes this so brilliant. Sharpless has a device that could lead to structures that bind tightly to proteins. In principle, this is one of the goals in the development of new drugs,' he says.

Future applications

In principle, the 'click chemistry' approach to drug design is applicable to almost any target. If it is possible to use a pure enzyme, the function of which can be measured easily, then the techniques are easier to apply. However, Finn stresses, 'the *in situ* approach has the virtue of being potentially useful when these conditions are not met. If everything works perfectly, one can even imagine administering a set of pieces of a drug to a patient and having the drug assemble itself at the desired site (e.g. a tumour) in response to the specific nature of the target. I emphasize that we are very, very far away from such an application.'

Further development work with other enzymes is now in progress and Finn confirms that the group is working collaboratively with many groups within and outside of Scripps, on a variety of diseases including AIDS, cancer, anthrax and Huntington's disease. 'It will be interesting to see how the Scripps scientists move this project forward. Minimally, one can be confident that interesting science will accrue. Very possibly, new drug candidates will ensue,' concludes Danishefsky.

Reference

1 Lewis, W.G. *et al.* (2002) Click chemistry *in situ*: Acetylcholinesterase as a reaction vessel for the selective assembly of a femtomolar inhibitor from an array of building blocks. *Angew. Chem.* 41, 1053–1057

New assays for measuring prions reassure FDA

Janet Fricker, freelance writer

Source plasma for manufacturing plasma derivative products has been exempted from US Food and Drug Administration (FDA) Guidance that recommends the deferral of blood donors who have lived for five years or more in Europe from 1980, in an attempt to exclude donors exposed to bovine spongiform encephalopathy (BSE) [1].

The exclusion ruling – due to be implemented in May 2002 – follows joint evidence presented to the FDA by Aventis Behring (King of Prussia, PA, USA), Bayer Corporation (Research Triangle Park, NC, USA) and Baxter Biosciences (Deerfield, IL, USA) on the capacity of their plasma-protein therapy manufacturing processes to remove prions, the infectious proteins responsible for BSE and Creutzfeldt–Jakob disease (CJD).

The companies – all members of the Plasma Protein Therapeutics Association

(PPTA) and participants in its Transmissible Spongiform Encephalopathy (TSE) Working Group – presented evidence on studies looking at the removal capacity of prions from manufacturing processes miniaturized to laboratory scale. The data demonstrates that many of the manufacturing steps involved in purifying proteins also have the ability to remove prions. The studies were made possible by the development of new, rapid and more practical *in vitro* assays for measuring prions, such as western blotting and conformation-dependent immunoassays (CDI).

Plasma is the source of many therapeutic proteins, such as anti-haemophilic clotting factor, immunoglobulins, albumin and fibrinogen, which are used in the treatment of shock, burns, immune deficiency disorders, cardiopulmonary bypass surgery, haemophilia and von Willebrand disease (http://www.plasmatherapeutics.org/plasma_therapeutics/plasma_protein.htm). Each year, more than one million people worldwide receive plasma therapies.

Risk of transmitting CJD

'Although there is only a theoretical risk that plasma products could transmit CJD, the plasma protein industry feels

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