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.

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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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obliged to apply the precautionary principle of doing everything we can to minimize that risk,' says Henry Baron, Senior Director of Medical and Scientific Affairs at Aventis Behring (Paris, France) and chairman of the PPTA TSE Working Group.

Currently there is no evidence that people with preclinical or clinical CJD, including the recently described variant caused by consumption of meat products derived from BSE-infected cows (vCJD), carry infectious prions in their blood or have transmitted them through blood or plasma products [1].

However, certain characteristics of vCJD differentiate it from classical CJD. For example, in patients with vCJD, unlike those with classical CJD, the pathogenic prion protein has been found in lymphoid tissues, such as tonsil and spleen [2], giving rise to currently unsubstantiated fears of blood infectivity carried by circulating lymphoid-tissue-derived blood cells.

To assess the theoretical risk, pharmaceutical companies have been developing assays to measure prion partitioning in protein purification. Historically, prion infectivity was measured in rodents by using end-point dilution titration and challenging back into the animals. However, because of the species difference, the passage of prions from humans to rodents requires prolonged incubation. The use of transgenic mice expressing a chimeric human–mouse prion protein was more efficient and speeded up the infection process [3] but incubation still took ~200 days.

Western blotting versus CDI

A recent study by Douglas Lee and colleagues at Bayer, demonstrating a direct correlation between partitioning of hamster prions determined by western blotting and infectivity determined by a bioassay in hamsters, established that western blotting can be used as an effective alternative to bioassays for tracking infectivity in plasma protein purification [4], and it can measure prion partitioning over a 4–5 log range.

The western blotting assay makes use of the fact that normal, cellular prion protein (PrP^{C}) undergoes significant alterations in both secondary and tertiary structure in its diseased state (PrP^{Sc}), affecting the physicochemical nature of the molecule. The PrP^{Sc} isoform is more resistant to proteinase than its normal counterpart, such that treatment with proteinase K (PK) results in the removal of only 90 amino acids from the N terminus, enabling its detection by western blotting [5].

While both Bayer and Baxter have used western blotting to measure prion partitioning, Aventis Behring have used CDI as their assay system [6]. CDI resulted from a research collaboration between Aventis Behring and 1997 Nobel laureate Stanley Prusiner (who elucidated the prion concept), and represents a major advance because it overcomes the need for protease digestion and thus can detect some strains of prions that are susceptible to protease digestion [7]. CDI is a time-resolved fluorescence (TRF) immunoassay in the format of an enzyme-linked immunosorbent assay that takes advantage of the profound conformational change between the normal, predominantly α-helical form of the prion protein and the pathogenic, predominantly β-sheet form. CDI can detect and quantify the pathogenic, β-sheet-folded prion by measuring differential antibody binding to the prion protein in a sample before and after denaturation.

Aventis Behring has been working to further improve the sensitivity of CDI. At the recent Institut Pasteur Euroconference *Viruses and New Emerging Agents in Biologicals: A Safety Approach* [8] the company presented data showing that CDI sensitivity can be improved 10–30-fold using the 'sandwich' method of immunodetection. A proprietary Aventis Behring monoclonal antibody specific to the human prion protein in both its normal and abnormal forms is used as a 'capture' reagent, replacing the chemical cross-linking reagent glutaraldehyde used in the original CDI. 'The result is that you have much less background noise giving a cleaner lower baseline from which to detect abnormal prions,' says Baron. A recent World Health Organization study presented at the PPTA TSE Workshop [9] distributed CJD and vCJD brain samples to 16 laboratories and found that the CDI was 1–2 logs more sensitive than western blotting. 'This increased sensitivity could be important in spiking studies for the assessment of prion partitioning,' says Baron, 'It could allow measurement of lower levels of prions, thereby demonstrating greater reduction factors for the manufacturing process.'

Spiking studies for prion removal, which proved key to the FDA's plasma exemption policy decision, involve down-scaling the manufacturing process to a laboratory scale and then spiking with an exogenous amount of diseased prion. Clearance data is found by comparing the amount of prion put in with the amount in the resulting fraction. In most studies, hamster prions are used to spike the samples because of the shortage of vCJD and sporadic CJD material. Studies by Aventis Behring and Bayer comparing classical CJD, vCJD and hamster prions showed that prion partitioning results are comparable for the different strains evaluated [10,11], suggesting that the hamster prion model is relevant for use in these spiking studies.

Another difficulty is that because prions have never been detected in human plasma, it is difficult to be certain of the exact physicochemical nature of the theoretical contaminant. To investigate whether purity of the spiking agent affects partitioning, Aventis Behring has evaluated the clearance of four different hamster prion preparations, ranging from the least purified form (crude brain homogenate) to the most highly purified

form (free molecular pathogenic prion protein). They found that the non-membrane-associated molecular spike partitioned differently from the three membrane-bound spikes. Removal is now being evaluated with two spiking agents – one membrane-bound and the other unbound, so that results can be expressed as a log range between the 'best case' and 'worst case' scenario for prion reduction [6]. Other companies, such as Bayer and ZLB (Bern, Switzerland), are beginning to evaluate purified forms of prion innocula as spiking agents.

Combining both methods

Although different companies have used different methods for prion detection, this might not represent a disadvantage. 'When you correlate Bayer's and Baxter's work on [the] western blot together with Aventis' work with CDI, this produced independent verification that was enormously reassuring to the FDA,' says Steve Petteway, Director of Pathogen Safety and Research at Bayer.

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Novel templates for rapid protein separation

Sharon Dorrell, freelance writer

Scientists are under pressure to provide innovative drug treatments fast. A new molecular imprinting technique developed at Aspira Biosystems (San Francisco, CA, USA) could speed up protein separation and, therefore, target molecule characterization. The technique, which is called ProteinPrint™, enables the generation of cavities complementary to a portion of a protein of interest. The cavities can be synthesized on tiny beads approximately 10 μm in diameter or on microarrays. The cavities selectively bind to protein that fits the mould, similar to the way in which antibodies bind antigen, but with the ability to distinguish even a single amino acid mismatch. Each bead can bind an estimated one million protein molecules, although the company is working on increasing the capacity of the beads.

Molecular imprinting

ProteinPrint™ is based on a process known as molecular imprinting, in which synthetic monomers are allowed to self-assemble around a template peptide to create a synthetic mould of the peptide. A signature sequence of amino acids acts as the template molecule. 'The signature sequence of a protein could be any portion of the protein that is unique to that protein,' explains Casey Lynch, President of Aspira Biosystems. The signature sequence might be derived experimentally or drawn from a database of predicted sequences. 'In our case, we usually use the C-terminal 7–9 amino acids, which provides a unique signature for 80–90% of proteins in the proteome and is easier to predict for novel proteins because of stop codons,' says Lynch.

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