A new opening for large-protein therapeutics

For decades, it has been accepted throughout the pharmaceutical industry that any compound larger than the 'bioavailability wall' of 500–600 Da would not be able to cross the lipophilic cell membrane into the cell. This has limited the development of therapeutic peptides to a maximum of approximately six amino acids in length, and ruled out protein therapy completely. However, Steven F. Dowdy (Howard Hughes Medical Institute and Washington University School of Medicine, St Louis, MO, USA) and his colleagues have now demonstrated that enzymes fused to part of a protein from the HIV virus can cross cell membranes while retaining their enzymatic activity *in vivo* in mice [Schwarze, S.R. *et al.* (1999) *Science* 285, 1569–1572].

It has been known for approximately ten years that the transactivating regulatory protein (TAT protein) from the HIV virus has an unusual ability to cross cell membranes without using receptors or transporters, or requiring ATP [Green, M. and Loewenstein, P.M. (1988) *Cell* 55, 1179-1188]. Although its exact mechanism is unknown, it has been shown that the protein transduction domain (PTD) of TAT opens a 'hole' in the cell membrane lipid bilayer, pulling anything covalently attached through it, before closing it again. This is a specific process that does not otherwise damage the cell. Dowdy's group pioneered a technique for fusing proteins to the PTD, which is an 11-residue sequence of largely basic amino acids. Although they have previously demonstrated the transduction of over 50 different fused proteins into cultured human and mouse cells, this is the first time this technique has been shown to work *in vivo*.

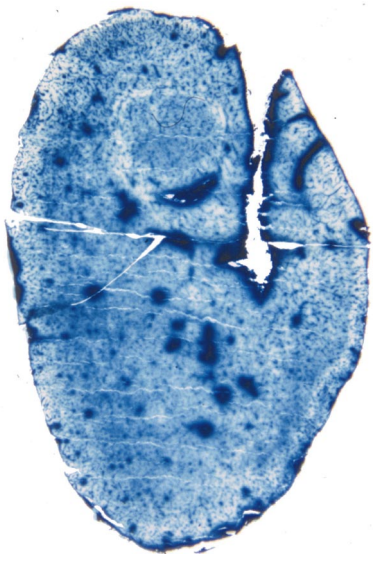

Figure 1. *Brain tissue from a mouse, obtained four hours after the animal was injected with the enzyme β-galactosidase attached to a protein transduction domain. The bacterial enzyme turns a clear X-Gal liquid a blue colour, revealing the enzyme's location.*

The group have demonstrated the efficacy of protein transduction *in vivo* using a fluorescence assay. C5BL/6 mice were injected intraperitoneally with either fluorescin isothiocyanate (FITC) covalently bound to PTD via a linker, or with free FITC as a control. This fluorescent dye, which has a molecular weight of 2000 Da, is normally too large to cross cell membranes. However, fluorescence was observed in all blood, spleen, muscle and brain cells in mice that had received PTD–FITC, whereas no fluorescence was seen in the control mice.

Retention of protein activity

To test the activity of proteins transduced in this way, the enzyme β-galactosidase (β-Gal; MW = 116,000 Da) was fused to the PTD with a leader sequence. Similar mice were injected either with this fusion protein or with control β-Gal fused to the N-terminal leader without the PTD. Tissue samples from the mice were tested for β-Gal activity using a standard X-Gal assay. X-Gal is a clear liquid that produces a blue precipitate when its galactose moiety is cleaved. A blue colour, indicating the presence of active β-Gal, was detected in the liver, kidney, lung, skeletal muscle and spleen of mice four hours after injection with PTD-β-Gal. Enzymic activity in brain cells also reached a maximum after four hours, indicating that the fused protein had crossed the blood–brain barrier (Fig. 1). By contrast, sections from control mice showed no β-Gal activity in the brain, heart or lung, and very little in the liver and kidneys. Hence, the fused protein, which denatured as it crossed the membrane, had been re-folded into active enzyme by the mouse chaperonins. This is all the more surprising as β-Gal is a bacterial protein, and most therapeutically useful proteins are of mammalian origin and so, presumably, would be re-folded more efficiently by human chaperonins. Stephen Schwarze (Washington University School of Medicine) suggested that the next step will be to transduce a protein that gives an interesting pharmacological response in an animal model.

Possible applications

The exquisite specificity of proteins has evolved over billions of years. A

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reliable method of delivering proteins into cells could therefore lead to the development of highly specific drugs. Dowdy's group has already demonstrated several possible therapeutic applications *in vitro*. They fused caspase-3, an apoptosis promoter, to the PTD and replaced the endogenous caspase cleavage sites with sites specifically cleaved by the HIV protease. When this was added to media containing Jurkat T cells, some of which had been previously infected by the HIV virus, only the infected cells were killed [Vocero-Akbani, A.M. *et al.* (1999) *Nat. Med.* 5, 29–33]. It should therefore be possible to generate similar protein chimeras that would be effective against other pathogens that are dependent on specific proteases. A similar technique has been used to selectively kill cancer cells. Transducing a PTD-linked tumour suppressor protein into cultured tumour cells induced cell-cycle arrest followed by apoptosis. Although cell-cycle arrest was also induced in normal cells transduced by the same tumour suppressor protein, these cells did not die.

Many questions must be answered before protein drugs based on this technology can reach the clinic. It is possible that the PTD might be immunogenic, although Dowdy believes that any such problem could be overcome. 'We are now working with synthetic PTDs. Each of these sequences is more efficient than the HIV PTD. It is very unlikely that all possible synthetic transduction domains will induce an immune response.' Although it could be very difficult to target a particular cell type using this technique, it is likely to enable the application of protein therapy to human disease.

Clare Sansom

Combinatorial approaches to chemistry and biology

The second Royal Society of Chemistry–BMCS Conference on *Combinatorial Approaches to Chemistry and Biology* took place at Churchill College (Cambridge, UK) on 30 June–1 July 1999. This international meeting of 250 participants was heavily oversubscribed, mainly because generous industrial support had enabled the organizers to assemble 17 multidisciplinary speakers, each experts in their respective combinatorial fields. A broad range of sciences that now involve combinatorial approaches were represented. Several recurrent themes also emerged that highlighted recent developments in the field, including knowledge-based library design, polymer-supported reagents (PSRs) and catalysts, and new methods for carbon–carbon bond formation.

Polymer supporting reagents

The impact of PSRs (e.g. chemical reagents such as oxidants, reducing or dehydrating agents and organopalladium catalysts, which are attached to a polymer and are not free in solution) in synthetic organic chemistry is already very notable, and it is clear that this is just the beginning of their application in solution-phase parallel synthesis. Graham Showell (Cambridge Combinatoria, Cambridge, UK) reviewed the status of PSRs in combinatorial chemistry using, for example, the impressive solution synthesis of epibatidine devised by Steven Ley's group (Cambridge University, UK). Epibatidine is an unusual pyridine derivative that was only discovered in 1992 on the skin of poisonous frogs and is of great interest because it is >200-fold more active than morphine as an analgesic [Habermann, J., Ley, S.V. and Scott, J.S. (1999) *J. Chem. Soc., Perkin Trans. 1* 10, 1253–1256]. David Drewry (GlaxoWellcome, Research Triangle Park, NC, USA) then described the PSR work of GlaxoWellcome's combinatorial technology team. Subsequently, Michael South (Searle Discovery Research, St Louis, MO, USA) presented the elegant work of Monsanto, who are developing multistage reactions that incorporate the versatility of PSR in many of its different guises (e.g. as reactants, reagent scavengers and as by-product traps). These three lectures emphasized the commonly accepted advantages of PSR in the synthesis of smaller libraries (<2000 components) such as the ease of chemistry validation, analysis, purification and scale-up. One of the major future objectives will be to increase the versatility and loading of these reagents, while decreasing their cost, so that they can be used more effectively in the multistage synthesis of drug-like molecules.

New technologies

Chris Abell (Cambridge University, Cambridge, UK) described the use of Atomic Force Microscopy to determine ligand-binding from libraries. Meanwhile, James McCann (Cambridge

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