DRUG DELIVERY

Engineering a smoother ride

Blood sugar levels for some diabetics can seem to imitate a roller-coaster ride. Tight regulation of blood glucose levels is vital to avoid the fatal long-term complications of diabetes. At present, injecting insulin frequently fails to provide such a degree of control. In the August issue of *Nature Biotechnology*, researchers at Eli Lilly report how they generated novel crystal forms of insulin, which can provide longer and smoother control of blood glucose.

In the 1930s, the concept of combining insulin with zinc and protamine (an arginine-rich basic protein) led to the development of the Neutral Protamine Hagedorn (NPH) insulin preparation, which gave improved sustained action for diabetics. NPH insulin, a microcrystalline suspension, is used today to provide medium-to long-term activity. However, the duration of action is too short to provide basal therapy with a single injection per day, and its pharmacodynamic profile has a large peak. Brader *et al.* sought to retain the favourable properties of NPH insulin, yet correct its pharmacokinetic shortcomings, by manipulating the release rate of an NPH-like formulation.

The researchers co-crystallized human insulin (HI) with the less-soluble insulin derivative octanoyl-N^{ε}-LysB29-HI (C8-HI) in the presence of zinc and protamine. By altering the ratio of the soluble HI and less-soluble C8-HI versions of insulin, they were able to build stable co-crystals that released insulin at a slower rate than standard formulations of NPH. Low crystal solubility in the interstitial fluid is essential for controlled delivery. In dogs with transient experimental diabetes, a single injection of the co-crystals provided a sustained control of blood glucose levels for 24 hours, with nearly ideal pharmacodynamics.

The lipophilic moiety does not interfere with the order of the hexamer building blocks of insulin crystals, and confers a proportionate decrease in aqueous solubility. Both HI and C8-HI are biologically active. The key to the superior release profile is the proportion of the octanoyl derivative relative to unmodified protein.

Could this strategy be applied to the delivery of other proteins? Because

HIV

Vif — a new therapeutic target?

Viruses have evolved various mechanisms to avoid recognition and destruction by the host immune system. By studying these mechanisms, we can identify new targets for drug development. Several strategies have been developed to combat the AIDS-causing human immunodeficiency virus-1 (HIV-1), but problems with resistance create continuing pressure for new drugs. A new study in *Nature* describes the identification of a human protein, CEM15, the function of which is suppressed by the HIV-1 virion-infectivity factor (Vif), leading to the production of infectious virus particles known as virions. Although it was known that Vif has an essential part in HIV replication, its precise functions have remained unclear until now.

The Vif-deficient virions that are produced by primary T cells are non-infectious, and these T cells, and certain T-cell lines, are referred to as non-permissive (NP). By contrast, other cell types are termed permissive (P), because they can produce infectious Vif-deficient virions. Cell-fusion experiments have shown that the NP phenotype is dominant over the P phenotype, implying that a factor in the NP cells can influence the production of infectious Vif-deficient virions. Malim and colleagues set out to identify this factor by using a complementary DNA subtraction strategy to compare NP and P cells. Subtracted cDNAs were used as probes in experiments to compare RNA expression in both NP and P cells. The authors identified a cDNA that corresponded to a gene that they named *CEM15*, and the transcript was identified in all of the NP cells that were tested. To test the function of CEM15, the protein was expressed in a permissive T-cell line that does not normally express the protein. Instead of producing Vif-deficient infectious virions, the resulting virus particles were non-infectious.

So what is CEM15? Analysis of the CEM15 protein sequence showed that it has marked homology to APOBEC1, a cytidine deaminase that specifically edits *APOB* mesenger RNA.

CEM15 seems to be responsible for the inability of NP cells to produce infectious Vif-deficient virions, which indicates that CEM15 is the cellular target of Vif. Because of its homology to APOBEC1, the authors speculate that CEM15 might affect Vif-deficient virions by means of interactions with viral RNA. Importantly, the CEM15–Vif interaction might prove to be an important therapeutic target for the development of new drugs to alter virus infectivity. Inhibiting Vif should allow the natural defence mechanism of CEM15 to take control of the infection. Continuing work includes identifying substances that bind to and inhibit Vif in the cell. But for now, the function of CEM15, and how Vif suppresses this function, remain to be determined.

Elaine Bell, Editor,
Nature Reviews Immunology

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conjugation of a lipophilic moiety to the protein might interfere with its conformation and render co-crystallization and solubility difficult to control, it is likely that the success of this approach will be determined on a case-by-case basis. Other sophisticated drug delivery technologies, including hydrogels, liposomes and microspheres, provide a matrix from which a drug can be released in a controlled fashion. However, these complicated formulations often reduce the efficacy of the therapeutic protein or render mass production of the drug difficult. Tweaking the drug itself, by altering the composition of the protein crystal lattice without losing efficacy, might be a simpler and more effective alternative.

Melanie Brazil

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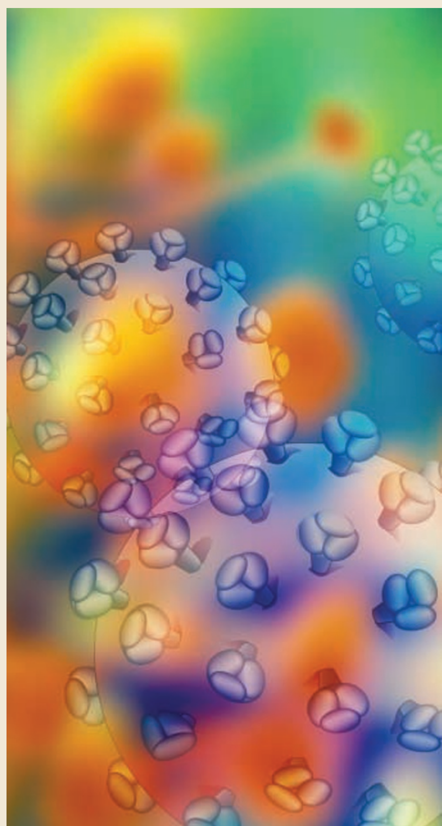

HIGH-THROUGHPUT SCREENING

Lighting up targets with reporter ribozymes

Most methods to monitor interactions between biological macromolecules, and to identify compounds that can disrupt these interactions, need one of the components to be labelled, or are not amenable to real-time detection, hindering their application in large-scale parallel screening. Now, by combining two classes of oligonucleotides — known as ribozymes and aptamers — Hartig *et al.* have developed a technology called 'reporter ribozymes', which can monitor macromolecular interactions in real time, and could potentially be compatible with the parallel screening of large compound or protein libraries.

Ribozymes are RNA molecules that can catalyse simple nucleic-acid-based reactions, such as RNA cleavage or ligation. RNA cleavage can be detected by attaching two tags to a substrate RNA oligonucleotide that is bound to the ribozyme — a fluorophore and a quencher that suppresses the fluorescence signal when close to the fluorophore. So, when the ribozyme catalyses the cleavage of the bound oligonucleotide, the fluorophore and the quencher are separated, which results in a fluorescence signal.

How can this cleavage be used to report protein interactions? This is where the aptamers — single-stranded nucleic acids that can fold into intricate globular structures capable of binding proteins with high potency and specificity — come to the fore. Linking the ribozyme to a protein-binding aptamer in such a way that the ribozyme cleavage reaction is dependent on whether the aptamer is bound to the protein or not allows the interaction between the protein and the aptamer to be monitored from the fluorescence signal due to the ribozyme activity. Other interactions of the protein — for example, with a small molecule — will influence the interaction between the protein and the aptamer, and so be detectable by a change in the fluorescence signal.

The authors explored several formats for this technology. In the first format, they coupled a naturally occuring aptamer to the HIV-1 Rev protein to a ribozyme in such a way that binding of Rev to the aptamer inactivated ribozyme cleavage, inhibiting the fluorescence signal. By contrast, in the second format, a Rev-binding aptamer that had been created previously was coupled to a ribozyme, such that binding of Rev would allow ribozyme cleavage, resulting in a fluorescence signal. Both formats were used to screen a 96-member library of antibiotics for molecules that could disrupt the binding of Rev to its aptamer, and both identifed three compounds as hits, one of which was subsequently shown to inhibit HIV replication, indicating that the protocol can generate drug leads.

To illustrate the potential for monitoring protein–protein interactions, the authors developed a third format, using an aptamer to α-thrombin that prevents cleavage of the ribozyme unless α-thrombin is bound. The decrease in fluorescence signal on addition of a protein inhibitor to α-thrombin, which disrupts the the ribozyme–α-thrombin interaction, could report the inhibitor–α-thrombin interaction in a concentration-dependent manner.

The third format is potentially compatible with high-throughput screens for small molecules that act as superior thrombin inhibitors. Moreover, in this case, the aptamer could be swapped for other aptamers to alternative protein targets. As approaches are being developed to create such aptamers for a broad range of proteins efficiently — and importantly, with the ability to selectively target specific domains, such as the protein active site — it seems that reporter ribozymes based on aptamers are likely to become increasingly useful as tools for drug screening.

Peter Kirkpatrick

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