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New PNA tool makes great antisense

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Peptide nucleic acids (PNAs) have been shown for the first time to be capable of forming a hybrid quadruplex with DNA, a structure composed of PNA_2–DNA_2. This finding, made by a research group lead by Bruce A. Armitage, Associate Professor of Chemistry at Carnegie Mellon University (http://www.cmu.edu/), also stands out because the PNA used to form the quadruplex was homologous, as opposed to complementary, to the DNA [1].

Scientists believe that these results provide further evidence that PNAs are likely to be useful for medical applications. 'This new PNA–DNA construct, and similar complexes, may potentially act as a repressor in diverse gene expression regulatory pathways, including oncogenes and genes involved in neuropathologies,' said Vadim V. Demidov, Senior Scientist at Boston University's Center for Advanced Biotechnology (http://www.bu.edu/cab/). 'They could also be used as biosensors for *in vitro* diagnostics or as building elements for complex nano-assemblies.'

Peptide nucleic acids

PNAs are oligonucleotide analogues in which the entire sugar–phosphate backbone has been replaced with a pseudopeptide [2]. These molecules, which were first reported in 1991, have attracted interest due to their high affinity for DNA and their resistance to degradation by nuclease and protease enzymes.

Previous studies have shown that PNAs can form a wide variety of complexes via Watson–Crick and Hoogsteen base pairing, including PNA–DNA and PNA–RNA duplexes and

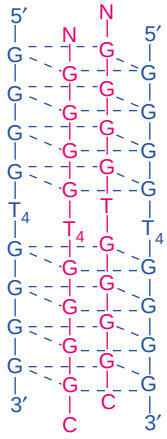

Figure 1. A guanine-rich peptide nucleic acid (PNA) strand forms a four-stranded hybrid quadruplex with the homologous guanine-rich DNA strand. PNA and DNA strands are shown in magenta and blue, respectively.

$PNA–DNA_2$, PNA_2–DNA, PNA_2–RNA and PNA_3 triplexes, said Demidov, a recognized expert in the field.

In the current study, the investigators were interested in the fact that DNA with a long stretch of guanine (G) bases can fold back on itself and form a stable structure. This structure, a G-tetrad, is stabilized by eight hydrogen bonds that involve both the Watson–Crick and Hoogsteen faces of the guanine bases. 'If four guanines from DNA could do this, then maybe we could design PNAs that had guanines in it that would bind to DNA in the same fashion,' explained Armitage.

However, it was far from clear at the outset whether this would occur. 'One of the things we've learned over the years working with PNA is never to expect anything,' said Armitage. 'It's kind of a strange molecule because it's part nucleic acid and part peptide and you never know which of those two properties are going to dominate.'

Quadruplex formation

The Carnegie Mellon researchers chose to target a DNA sequence present in telomeres of the ciliated protozoan *Oxytricha nova*. In solution, a single strand of this sequence, 5′-G4-T4-G4-3′, forms a hairpin structure that binds to another strand to form a dimeric G-quadruplex [3]. 'What we do is add our homologous PNA to that solution and then we heat it to 90°C and that causes the DNA quadruplex to open up,' Armitage explained. 'When we cool it, the PNA is able go in and form the hybrid quadruplex that has four strands in it, two PNAs and two DNAs.' Fluorescence resonance energy transfer indicates that the two DNA strands are parallel to each other, the two PNA strands are also parallel to each other, and the 5'-termini of the DNA strands align with the N-termini of the PNA strands (Fig. 1).

Why does a four-stranded quadruplex form in their experiment? It is probably more stable because a four-stranded structure forms eight G-tetrads and has 64 hydrogen bonds, as opposed to a two-stranded structure that forms four G-tetrads and thus only has 32 hydrogen bonds. At room temperature, they believe a one-to-one, PNA–DNA quadruplex forms.

These tetrad structures are stronger than DNA complexes that form through Watson–Crick base pairing as each tetrad has eight hydrogen bonds whereas a G–C (cytosine) pair only has three, Armitage said. In addition, it is thought that other factors contribute to their stability, such as the fact that PNA

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lacks the negative charges that are present along the DNA backbone.

Currently, they are working to characterize the hybridization of short PNAs to G-rich DNA. 'As we learn more about the range of quadruplexes that PNA can form, then we can start to think rationally about targeting actual biological systems,' Armitage said.

Therapeutic approaches

There is evidence that a G-quadruplex, which forms upstream of the c-Myc gene within chromosomal DNA, must be unfolded in order for a transcription factor to bind and activate gene expression [4]. 'If you have something that's bound very tightly to the guanine-rich sequence, then it could prevent expression of the oncogene,' Armitage said. It is possible that a homologous PNA could either hybridize with the G-rich strand to form a quadruplex or to the C-rich strand to form a duplex – both of which would theoretically block transcription factor binding and c-Myc expression.

PNAs have many features, including high DNA and/or RNA-binding affinity, recognition specificity, biochemical stability and remarkable strand-invasion ability, that suggest their potential as therapeutics. However, work to date, including the current study, has occurred in a simplified model system. 'Hence, sequence and structure specificity of quadruplex-forming PNA ligands, and workability of their isothermal binding to corresponding nucleic acid targets at physiological temperatures, have to be thoroughly addressed for the use of these G-rich PNAs in biologically relevant complex systems,' said Demidov. 'Some more difficulties, such as poor solubility, target accessibility and tissue-specific or intracellular delivery, could also be encountered.'

References

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4 Siddiqui-Jain, A. *et al.* (2002) Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11593–11598

News in brief

Targets and Mechanisms

Multi-talented enzyme

The structure of an enzyme that is key to the initiation of many biochemical pathways has been elucidated by researchers at St Jude Children's research hospital (http://www.stjude.org) [1]. A group led by Brenda Schulman revealed that the enzyme uses two different parts of its own structure to juggle four different molecules as it completes three different reactions.

Vital cell processes are switched on by ubiquitin-like proteins such as NEDD8, which are activated by E1 enzymes to co-ordinate specific functions and ensure that they take place at the right time. The activation of ubiquitin-like proteins is actually a complex series of reactions that begins when a specific E1-activating enzyme brings together a ubiquitin-like protein and an E2 escort molecule. E2 escorts the ubiquitin-like protein to its pre-assigned target molecule for chemical modification, triggering a specific cellular activity.

Structural characterization of the E1 enzyme for NEDD8 has helped to explain how E1 manages to complete the three different reactions required to link NEDD8 to its E2 escort – they occur at distinct regions of the enzyme.

It is thought that this knowledge could help to explain the complex command and control systems that are disrupted in disease. For example, the influenza virus hijacks a ubiquitin-like protein so that it cannot undergo normal activation by an E1 enzyme. This helps the virus to hide from the immune system. 'The more we learn about how these pathways are controlled, the more likely it is that we'll understand how to fix them when they're disrupted,' stated Schulman.

1 Walden, H. *et al.* (2003) Insights into the ubiquitin transfer cascade from the structure of the activating enzyme for NEDD8. *Nature* 422, 330–334

AD and PD: a new link

The two most common neurodegenerative diseases have been linked at the molecular level. Researchers at the University of Pennsylvania School of Medicine (http://www.med.upenn.edu/) have discovered that a protein implicated in Parkinson's disease (PD) can induce the aggregation of a protein associated with Alzheimer's disease (AD) [2]. This new connection could explain why patients with one disease are more likely to show signs of the other.

Both PD and AD are characterized by amyloid lesions caused by clumps of tangled proteins. The protein α-synuclein is one such contributory factor in PD, in which it binds to itself enabling the formation of Lewy bodies. The tau protein has a similar role in the onset of AD, but is larger and requires cofactors to initiate the formation of fibrous clumps. Both proteins are naturally abundant in the brain, where they have distinct functions: tau has a role in microtubule stabilization, whereas α-synuclein is involved in regulating communications at the synapse.

Beginning with *in vitro* studies, the researchers found that α-synuclein interacted

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