Synthesis and Use of Modified Peptide Nucleic Acids for Visual Detection of DNA

[(S,S)-Trans-1,2-Cyclopentane Diamine-Modified and Gamma-Lysine-Modified-Peptide Nucleic Acids as Probes for Nucleic Acid Detection: Synthesis and Application]

Description of Technology:

Modification of peptide nucleic acids (PNAs) by the incorporation of trans-1,2-diaminocyclopentane into the PNA provides for more highly sensitive and selective detection of DNA and RNA sequences. The compounds disclosed in this invention have the potential for use in the development of nucleic acid detection kits for various pathogens.

Genomic analysis can provide key diagnostic information about the presence of pathogens or disease. At the present time DNA-based devices, such as DNA microarrays, are used for such an analysis. However, most of these analyses require the amplification of the DNA under investigation. Replacing the DNA probe with peptide nucleic acid (PNA) greatly facilitates the DNA detection because the binding strength of PNAs to complementary DNA is stronger than DNA binding to complementary DNA. Strong binding between PNA and DNA can eliminate the need for prior DNA amplification. Furthermore, PNAs bind to DNAs under low salt conditions which disfavor formation of double-stranded DNA. By operating under low salt conditions, DNA can be denatured to ensure that target sequences are available for binding to a PNA probe. In addition, PNA is a synthetic molecule that cannot be broken down by enzymes, so the stability of PNAs allows for a device with a longer shelf life than one made from DNA. This technology also permits the presence of complementary DNA to be detected by eye (i.e. without instrumentation).

Applications:

The PNAs that have been synthesized in this invention may be adapted for detecting an infectious agent, such as anthrax, avian flu, severe acute respiratory syndrome (SARS), human papilloma virus (HPV), or human immunodeficiency virus (HIV). Other applications include:

- DNA analysis in biochemical research
- Chiral synthesis
- Diagnosis of pathogens in the environment
- Diagnosis of disease

Advantages:

- Peptide nucleic acids do not need refrigeration
- Binding to complementary DNA can be detected by eye
- Complementary double-stranded DNA can be detected without prior dissociation

Development Status:

- PNA backbone structure has been improved by incorporation of cyclopentane rings in the PNA backbone
- An improved synthesis of cyclopentane-containing PNA has been developed
- Visual detection of anthrax DNA has been demonstrated

Inventor:

Dr. Daniel Appella (NIDDK)

Publications:

Practical synthesis of trans-tert-butyl-2-aminocyclopentylcarbamate and resolution of enantiomers. Xu, Q., Appella, D.H. J Org Chem. 2006 Oct 27;71(22):8655-7. [Pubmed reference]

Gamma-substituted peptide nucleic acids constructed from L-lysine are a versatile scaffold for multifunctional display. Englund, E.A., Appella, D.J. Angew Chem Int Ed Engl. 2007;46(9):1414-8. No abstract available.

Patent Status:

DHHS Reference No. E-308-2006, a provisional application has been filed.

Licensing Contact:

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Collaborative Research Opportunity:

The National Institute of Diabetes and Digestive and Kidney Diseases is seeking parties interested in collaborative research directed towards developing this technology towards production. Please contact Dr. Daniel Appella at, appellad@niddk.nih.gov or Rochelle S. Blaustein at Rochelle.Blaustein@nih.gov for more information.

Research Focus and Selected Publications for Principal Investigator

Daniel Appella, Ph.D., Laboratory of Bioorganic Chemistry

Dr. Appella's laboratory uses synthetic organic chemistry to create new molecules with unique biological activity. Each molecule has the potential to be developed into a new strategy for diagnosing or treating a disease. Dr. Appella's research involves synthesizing molecules, molecular modeling and biophysical techniques to study the synthesized molecules we make. Dr. Appella's group also collaborates within the NIH and other institutions to study the biological effects of the molecules *in vivo*.

Peptide nucleic acids (PNAs) consist of an aminoethylglycine backbone to which nucleobases are attached. These molecules are very flexible, yet they bind to complementary DNA and RNA with high affinity and sequence specificity. There have been numerous biochemical and biomedical applications of PNAs, however there is no general strategy for functionalizing or preorganizing this class of molecules while preserving the binding properties to complementary nucleic acids. Dr. Appella's explores the incorporation of carbocyclic rings and sidechains into the backbone of peptide nucleic acids (PNAs) in order to improve binding to oligonucleotides and provide the basis for design of PNA-based sensors. Proper rigidification of PNAs could significantly enhance their binding properties to DNA and RNA, and improve many of the diagnostic techniques that rely on PNA. Through a combination of molecular modeling techniques, incorporation of a cyclopentane ring into the ethylenediamine portion of a PNA has been found to significantly improve its binding to DNA and RNA. The generality of this modification in several different PNA sequences is under investigation. Future work will place other carbocyclic rings and sidechains into PNAs and will develop new diagnostic techniques based on our modified PNAs.

1. Englund EA, Xu Q, Witschi MA, Appella DH PNA-DNA duplexes, triplexes, and quadruplexes are stabilized with trans-cyclopentane units. *J Am Chem Soc*(128): 16456-7, 2006. [Full Text/Abstract]

2. Hara T, Durell SR, Myers MC, Appella DH Probing the structural requirements of peptoids that inhibit HDM2-p53 interactions. *J Am Chem Soc*(128): 1995-2004, 2006. [Full Text/Abstract]

3. Myers, M. C.; Wang, J.-L.; Iera, J. A.; Bang. J.-k.; Hara, T.; Saito, S.; Zambetti, G. P.; Appella, D. H. A New Family of Small Molecules to Probe the Reactivation of Mutant p53 *Journal of the American Chemical Society*(127): 6152, 2005. [Full Text/Abstract]

4. Pokorski, J. K.; Nam, J.-M.; Vega, R. A.; Mirkin, C. A.; Appella, D. H. Cyclopentane-modified PNA Improves the Sensitivity of Nanoparticle Based Scanometric DNA Detection *Chemical Communications*: 2101, 2005. [Full Text/Abstract]