New Multilabel Fluorescent Groups for Increased Sensitivity of DNA Detection

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FINAL PROGRESS REPORT

GRANT # 31507LS - YIP

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STATEMENT OF THE PROBLEM STUDIED

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Our approach to investigating these problems has focused in part on evaluation of, and strategies for improvement of, sequence selectivity in nucleic acid recognition. We believe it is critical that molecular strategies be developed for obtaining as high selectivity as possible, so that differences as small as single point mutations can be identified in application. This work has involved making thermodynamic and kinetic measurements of nucleic acid hybridization, and has also involved the synthesis and evaluation of modified DNA structures which have improved selectivity.

The second focus has been on developing new molecular fluorescent tagging strategies for small oligonucleotides. The goal here has been to increase sensitivity and signal-to-noise in detecting a probe bound to a target nucleic acid. We originally envisioned some dual-label approaches to increasing signal-to-noise; however, more success has been forthcoming in increasing sensitivity. This work has involved synthesis of novel fluorescent molecules and developing strategies for increasing overall brightness of a given probe as evaluated by fluorescence spectrometry.

SUMMARY OF RESULTS

Our progress over the past three years in development of improved strategies for sequence discrimination has been nothing short of spectacular. Early work evaluated the use of circular oligonucleotides for recognition. After consideration of kinetics and thermodynamics of binding we confirmed our earlier finding that such modified DNAs can have much higher sequence discrimination than traditional linear probes (see graph below, for example). This work also led to useful information on why these probes are so successful, and thus give clues as to how selectivity can be improved yet further in the future. We then took some of this knowledge and designed a further molecular strategy for increasing sequence discrimination: the addition of crosslinks between triplex-forming oligonucleotides (see below). This work led to the development of one compound that displayed the ability to bind a predicted sequence 100 million times better than the same sequence with a single base changed (see graph below). This is the highest mismatch discrimination ever observed in a DNA-binding molecule.
Strategy for crosslinking two triplex-forming strands by engineering a disulfide bond into the DNA.\(^8\)

Comparison of mismatch discrimination by three types of oligonucleotides. The bicyclic case is a circular oligonucleotide with an added disulfide crosslink as shown above.

Other related work has extended this mode of recognition to RNA strands as well as DNA,\(^2\) and has increased the number of natural target sequences available for triplex binding.\(^9\) We have also better defined how small changes in structure can effect the recognition properties of such molecules.\(^3,4\) This work has gained international recognition, and we have recently written a number of reviews on such topics.\(^5,6,10,11\)

We have recently also had a good deal of success in development of new fluorescence methods for labeling small synthetic oligonucleotides.\(^13\) Our goals here are to find ways to label synthetic oligonucleotides as brightly as possible. We discovered early on that common fluorophores such as fluorescein and rhodamine could not be used in this application because they self-quench when placed
more than once in a short DNA. We therefore developed a new strategy for increasing fluorescence, in which excimer-forming groups are placed at adjacent sites in the DNA. This excimer strategy allows for increasing fluorescence intensity with addition of multiple fluorescent tags. The alpha-pyrene nucleoside below has been very successful for use as such an excimer label.

![Structures of new pyrene nucleosides developed under ARO funding in late 1995.](image)

Interestingly, the beta-pyrene nucleoside, also an excimer-forming fluorescent tag, may also be useful in improving recognition properties of nucleic acid probes. We recently showed, for example, that addition of a single beta-pyrene to a small strand increases binding affinity to a large degree (Guckian, et al., *J. Am. Chem. Soc.* 1996, 118, 8182) and we believe it also possible that discrimination will also be improved. This work is underway.

Finally, we have (in a different project) developed a new approach to synthesis of long DNA strands, which we term "rolling circle DNA synthesis" (D. Liu et al., *J. Am. Chem. Soc.* 1996, 118, 1587). We have now shown (P. Paris, work in progress) that this method can be used to synthesize long strands of multiply fluorescent-tagged DNA. We propose to use this (in future work) to label short, selective oligonucleotides very brightly (see below), so that very sensitive probes can be developed. These probes will be used to identify genes by fluorescence microscopy.
Proposed new approach for labeling a short hybridizing oligonucleotide with many fluorescein or rhodamine fluorophores. A short primer attached to the oligonucleotide is extended by rolling circle synthesis, incorporating fluorophores into the growing strand. This long fluorescent "tail" (thousands of nucleotides in length) is a simple repeating sequence and is designed not to bind to the target.

References
The references cited above are as listed in the "list of manuscripts" below.
LIST OF MANUSCRIPTS PUBLISHED IN THE GRANTING PERIOD*


*(only those papers which acknowledge ARO support are listed)
SCIENTIFIC PERSONNEL AND DEGREES

Graduate students supported:

  Pamela L. Paris, Squire Rumney IV. Degrees awarded: none; however, S. Rumney is currently writing his thesis.

Postdoctoral associates supported:


  Dr. Rex X.-F. Ren. Current position: Postdoctoral associate, Columbia University (with K. Nakanishi)

INVENTIONS AND TECHNOLOGY TRANSFER

A novel fluorescent DNA nucleoside was first developed in 1995, with continuing study in 1996 and beyond. This is a molecule in which pyrene, a well-known fluorophore, is incorporated into a nucleoside-like structure (see refs. 12 and 13 above). A patent is currently being applied for, and licensing of the structure and its applications is under discussion with a number of companies.