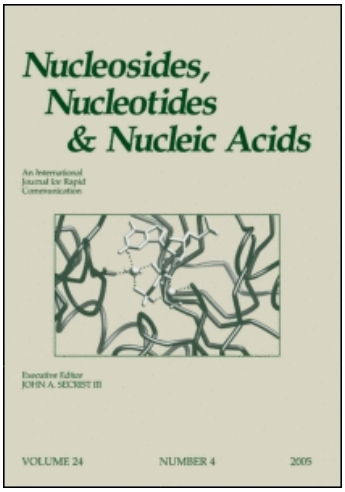

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SYNTHESIS AND HYBRIDIZATION CHARACTERISTICS OF OLIGODEOXYNUCLEOTIDE-ALKALINE PHOSPHATASE CONJUGATES

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Abstract: Modified oligodeoxynucleotides, 20 to 30 bases in length and containing "linker arm" bases, are chemically synthesized, then attached covalently to alkaline phosphatase in a 1:1 conjugate through a 19 atom spacer. Such conjugates hybridize rapidly and selectively to complementary sequences similar to unmodified oligomers, and allow colorimetric and fluorogenic detection of hybrids.

The most common methods for non-isotopic detection of nucleic acid hybrids are indirect systems which use either avidin-enzyme complexes to detect biotinylated DNA[1,2] or antibody-enzyme complexes to detect antigen-labelled DNA[3]. Some conceptual improvement was made by directly attaching alkaline phosphatase or horseradish peroxidase to double-stranded DNA directly[4]. Non-isotopic detection of oligodeoxynucleotide probes has been largely limited to chemical[5] or enzymatic[6] end-labelling with biotin.

Our laboratory has earlier reported synthesis of biotinylated or fluorescent probes via chemical synthesis of "linker arm" oligonucleotides[7]. This method, which provides modified thymine bases with a linker arm attached to C-5 at selected sites, allows incorporation of unique functionalities (amines, carboxylic acids, etc.) into oligomers. Using amine linker arms, we have recently conjugated alkaline phosphatase directly to oligomers[8]. Such conjugates are easily purified. Isolated products are 1:1 oligomer/enzyme mole ratio,

and retain full enzymatic activity. Relative to unmodified 32P-labelled oligomers, such conjugates exhibit similar stringency requirements for time, temperature, and buffers when hybridized against membrane-bound target. Kinetic studies using fluorogenic 4-methylumbelliferone phosphate as a substrate indicate that a 22mer conjugate achieves 50% of its maximum hybridization in 2.5 minutes at 10 nM probe when hybridized at 50° C in 5xSSC, 1% SDS; these results are identical to kinased oligomer. Although such conjugate probes can be detected by fluorescence, standard dye deposition detection using NBT/BCIP substrates offer good sensitivities as well. Hybridizations against dilution series of membrane-bound targets (plasmids and genomic DNA) give a colorimetric sensitivity limit of down to 0.5-1 attomols (about 500,000 molecules), or less than 5 pg of 6 Kb plasmid, in 15 minute hybridizations. No backgrounds from ug amounts of heterologous DNA (human, E. coli) can be detected. Relative to optimized hybridization with 32-P kinased oligomers, colorimetric detection of such conjugates has less background and is 2-5 times more sensitive, with no changes in hybridization protocols.

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