Abstract

Modifed oligonucleotides are routinely employed as bioanalytical probes for use in diagnostics; however, a major limiting factor in oligonucleotide-based diagnostics is poor cellular uptake. This can be overcome by covalent attachment of a cell penetrating peptide. Diels-Alder cycloaddition is an attractive methodology for oligonucleotide peptide conjugation; the reaction is fast and chemoselective and the reaction rate is greatly enhanced in aqueous media.

An oligonucleotide sequence has been derivatised with a series of dienes at the 5'-terminus, through chemical synthesis of a series of unique dienyl modified phosphoramidites. Investigation into the effect of diene type on the efficiency of conjugation to a maleimido derivatised Tat peptide derivative, *via* the Diels-Alder cycloaddition, has been performed. The optimal diene for biomolecule conjugation was found to be cyclohexadiene in the conjugation of oligonucleotides to a cell penetrating peptide *via* Diels-Alder cycloaddition for the first time.

An oligonucleotide sequence has also been derivatised with cyclohexadiene internally; Diels-Alder cycloadditions of these oligonucleotides to a maleimido derivatised Tat peptide derivative were also successful. However, oligonucleotide conjugation to Tat peptide *via* Diels-Alder cycloaddition of fluorescently labelled oligonucleotides for visualisation in cell studies has not been as successful as for unlabelled oligonucleotides.

Generation of a biocatalytic DNA sequence for the acceleration of Diels-Alder cycloadditions has been attempted. Through a SELEX-type process, a DNA sequence has been isolated for experimental determination of its potential as a biological catalyst for Diels-Alder cycloadditions.

Oligonucleotide conjugation to Tat peptide *via* gold nanoparticles has been achieved. Chemical synthesis of a dithiolated, long-chain PEGylated ligand allowed covalent attachment of Tat peptide to gold nanoparticles. Bifunctionalisation of gold nanoparticles with oligonucleotides and this ligand, followed by covalent attachment of Tat peptide generated the desired conjugate. The conjugates have been shown to hybridise successfully with the complementary oligonucleotide sequence, thereby displaying potential for their use as bioanalytical probes. Methods for quantification of both oligonucleotides and Tat peptide conjugated to gold nanoparticles, by enzyme hydrolysis, have been developed.