Proceedings of
Humboldt Kolleg:
SYNERGY, NETWORKING AND
THE ROLE OF FUNDAMENTAL
RESEARCH DEVELOPMENT
IN SOUTH EAST ASIA
in conjunction with:
THE INTERNATIONAL CONFERENCE
ON NATURAL SCIENCES (ICONS) 2011
SYNERGY, NETWORKING AND THE ROLE OF FUNDAMENTAL RESEARCH DEVELOPMENT IN ASEAN
in conjunction with:
THE INTERNATIONAL CONFERENCE ON NATURAL SCIENCES (ICONS) 2011

SYNTHESIS AND COVALENT ATTACHMENT OF A METHYLENE BLUE DERIVATIVE TO A TRIPLE HELIX FORMING OLIGONUCLEOTIDE – THE WAY TO NEW ANTICANCER DRUGS

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ABSTRACT - Phoactive methylene blue (MB) is a cationic planar chromophore which is able to interact with double and triple helical DNA. Thus on its applications can be used in the development of a new drug that specifically targets a DNA sequence. Covalent attachment of the MB to the DNA through a flexible heptamethylene linker has been accomplished, enabling the defined positioning of the dye moiety at specific sites of the DNA strands by forming a triple helix. The structure of MB itself does not provide a suitable functional group for a direct attachment to an oligonucleotide. Thus, the MB derivative, 3-N-(4-carboxybutyl)-ethylamino-7-dimethylaminophenazo-thiuranum chloride was synthesized. The attachment of the MB derivative to a 3'-aminoolkylated oligonucleotide was accomplished by the activation of the carboxyl group with a yield from 40%-70%. A precipitation step using ethanol was performed before HPLC purification in order to remove the excess of activated MB ester. Unreacted oligonucleotide was identified in the chromatogram from its absorbance at the typical wavelength for nucleic acids and its lack of absorbance at 660 nm. The conjugate products bearing the MB moiety were recognized from the additional absorbance at around 660 nm. Another confirmation comes from the MS data. 1D 1H NMR spectra of the MB-oligonucleotide conjugates as well as the non-conjugated 3'-aminoolkyl-oligonucleotide in H2O are acquired to compare the oligonucleotide before and after conjugation with MB.

Keywords: methylene blue, DNA recognition, oligonucleotide conjugate

I. INTRODUCTION
Triple helical nucleic acids have been known for almost fifty years but interest in these structural variants has considerably enhanced by some recent findings on the existence of triple-like structures in vivo.1-3 Triple helix formation has also been found to offer the basis for numerous site-specific manipulations on duplex DNA. Appropriately designed third strand oligonucleotides targeting a duplex to form a triple structure might be used to control gene expression by repressing the transcription step, to modulate the sequence-specificity of DNA-binding drugs or to selectively alter the sites of protein activity. Thus, many efforts have been dedicated to modify the third strand for particular purposes, e.g., by covalent attachment of certain ligands to the third oligonucleotide strand. Sequence-specific damage and cleavage of DNA strands employing photogenerated or/and intercalators tethered to third strand oligonucleotides have also been reported.4-6

Methylene blue (MB), a phenothiazinium dye, is a promising ligand that has been shown to interact with DNA and is able to cause a strand cleavage after photostimulation by generating singlet oxygen.7-9 This photostimulation process on MB might find its application as a noninvasive therapeutic tool in modern photomedicine, thus making this compound to be of big interest.10 In order to specify the preferred MB binding location, which in turn may lead to its potential use in the site-directed damaging of DNA targets, we have synthesized a MB-oligonucleotide conjugate where a MB derivative was covalently coupled to a 3'-aminoolkyl-modified DNA oligonucleotide third strand by a