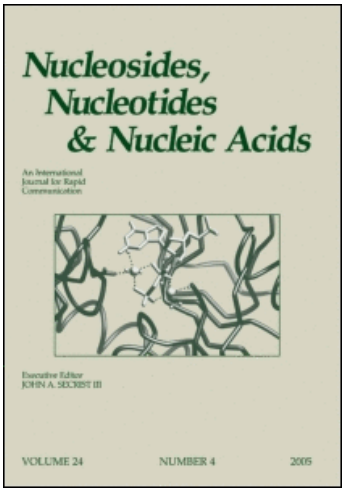

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A NEW FACILE METHOD FOR SPIN-LABELING OF OLIGONUCLEOTIDES

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ABSTRACT

A new facile method for spin-labeling suitable for DNA and RNA oligonucleotides is presented. The nitroxide 3-ethenyl-2,2,5,5-tetramethyl-pyrrolin-1-yloxy was directly introduced during automated solid-phase synthesis by a Pd(0) cross coupling reaction. The main advantages of this procedure are the small amount of spin-label needed for the derivatisation of the oligonucleotide and the high coupling efficiency on the solid phase.

INTRODUCTION

RNA and DNA are conformationally dynamic molecules. Especially RNA exists in a great variety of different secondary and tertiary structures. Furthermore both molecules form DNA/RNA-protein complexes, which play an important role in biological systems.

The investigation of their structure and the correlation with their biological function is a main subject in nucleic acid and pharmaceutical research. Along with UV-, CD- and NMR-spectroscopy, Electron Paramagnetic Resonance (EPR) has evolved as an appropriate method for gaining information about structural and dynamic parameters of nucleic acids and proteins (1–5). The main advantage of EPR-spectroscopy is the possibility of determining motions which occur in a timescale from millisecond to nanosecond. EPR possesses the additional advantage

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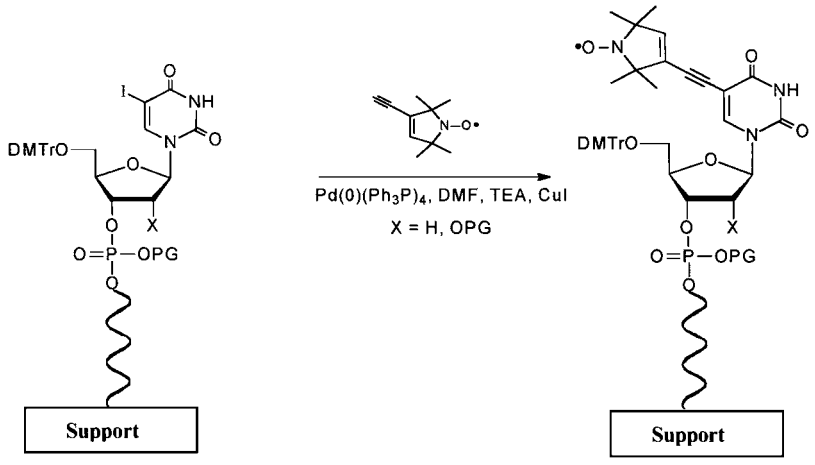
that very small quantities of sample are required compared to other of spectroscopic methods. A limitation in applying EPR spectroscopy to the study of nucleic acids is the absence of a natural unpaired electron. Stable radicals (spin-labels) have to be incorporated into the oligonucleotide at definite positions. The motion of this single reporter molecule or the interaction between two spin labels can be correlated with the motion of the oligonucleotide or the interspin-distance either (6).

RESULTS AND DISCUSSION

Different spin-labels have been attached to oligonucleotides by enzymatic or chemical means. We have chosen the nitroxide 3-ethenyl-2,2,5,5-tetramethyl-pyrrolin-1-yloxy as spin-label because of its rigidity and the possibility of attaching it covalently to the iodinated 5-position of uridine or cytidine by means of palladium(0)-catalyzed reactions.

Former publications described the incorporation of label 3 into DNA by preparing a labeled pyrimidine-phosphoramidite, which was then incorporated into the oligonucleotide (7). The synthesis of the phosphoramidite is a multi-step reaction, which affords the product in an overall yield of 47% starting from 5-iodo-2′-deoxyuridine and only 20% starting from 5-iodo-2′-deoxycytidine. The coupling efficiency on the synthesizer using this building block is significantly lower than typically for DNA synthesis.

Herein we present a new method of spin-labeling of oligonucletides during automated solid-phase synthesis by on-column derivatisation. The attachment of the spin-label is accomplished by a Palladium(0)-cross coupling directly on the column during standard automated synthesis analogues to a procedure published by Khan and Grinstaff (8). By using this method only a very small amount of spin-label 3 is consumed (less than 1 mg per labeled nucleotide) (Scheme 1).



Scheme 1. Spin-labeling during automated solid-phase synthesis.

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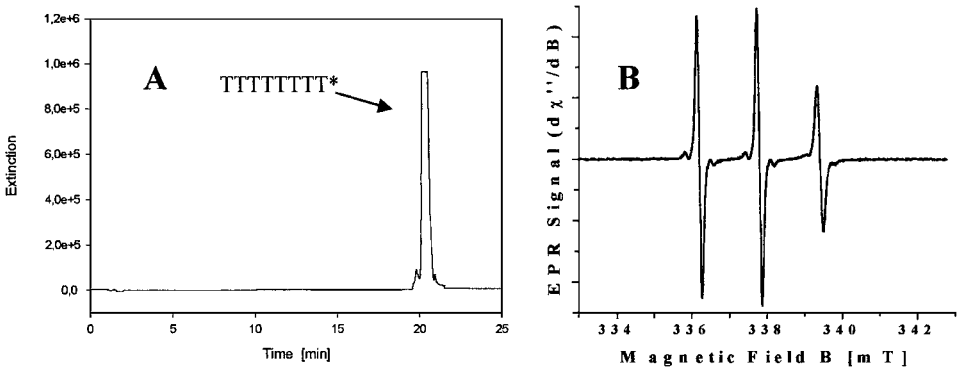

Scheme 2. (A) Preparative HPLC spectra and (B) EPR-spectra at 9.48 GHz (290 K) in phosphate buffer (140 mM NaCl) of a single stranded octa-T sequence.

The oligonucleotide syntheses were performed on an Eppendorf D300+ synthesizer in the 3′- to 5′-direction using standard phosphoramidite chemistry (1 μmol scale). A 0.1 M solution of 5′-DMTr-3′-cyanoethyl-N,N′-diisopropyl phosphoramidite-2′-deoxy-5-iodouridine in acetonitrile was installed on the synthesizer at an additional postion. The syntheses were stopped after incorporation of 5-iodo-2′-deoxyuridine-phosphoramidite or 5-iodouridine-phosphoramidite without deprotecting the 5′-hydroxyl group. The column was removed from the synthesizer while the anhydrous conditions were maintained by purging with N_2.

Subsequently a solution of $Pd(Ph_3P)_4$ (3 mmol), CuI (3 mmol) in 200 ml DMF:Et_3N (3.5:1.5) was prepared. Deoxygenating this solution turned out to be very important for the successfull Pd(0) cross coupling reaction. Finally the spinlabel (6 mmol) was added and the deep red solution was given onto the column using two syringes. After two hours the column was washed with DMF:Et_3N (20 ml). The column was reinstalled on the synthesizer and after performing two washing procedures with acetonitrile the solid-phase synthesis was resumed. Cleaveage from the solid-support and removal of the base-protecting group were performed at room temperature with 32% ammonia over 24 h. In case of RNA oligonucleotides the 2′-protecting group was removed with TBAF-solution. The crude RNA oligomer was precipitated with BuOH at -20°C. HPLC purification was accomplished using reverse phase for DNA and anion exchange chromatography for RNA. All oligonucleotides were characterised by MALDI-TOF-MS.

By using this method it is possible to synthesize many different EPR active probes in a time saving procedure.

As the incorporation of spin-labels into proteins is well established, this method could be a promising method to investigate distances, structure and dynamics in DNA/RNA-complexes.

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