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TRIFLUOROMETHYLDIAZIRINE-CONTAINING dUTP : SYNTHESIS AND APPLICATION IN DNA/PROTEIN CROSSLINKING

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ABSTRACT: The 5-[N-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoyl)-3-aminoallyl]-2'-deoxyuridine-5'-triphosphate was synthesized via acylation of 5-aminoallyl-2'-deoxyuridine-5'-triphosphate with 4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoate N-hydroxysuccinimide. It was used for the preparation of 30 bp ATFMD-DNA coding for promoter sequence. UV-Irradiation (365 nm) of the specific complex of this duplex and *E.coli* RNA polymerase leads to the effective crosslinking DNA with all protein subunits.

INTRODUCTION

Photochemical crosslinking is a powerful tool for the study of nucleic acid/protein interactions. The incorporation of modified nucleotides with photoactivatable groups into the nucleic acid molecules is one of the most modern approaches: the irradiation generates highly reactive particles forming covalent bonds with adjacent proteins. Several chemical groups for DNA/protein photocrosslinking (mostly arylazides or thio-) have been used^{1,2}. Photoreagents on the basis of aryl(trifluoromethyl)diazirine (ATFMD) possess some important advantages over the other photoactivatable compounds³ but up to now there were only a few examples⁴⁻⁶ of the application of ATFMD-groups for DNA/protein crosslinking.

In this work we describe the preparation and the application of 5-[N-(4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoyl)-3-aminoallyl]-2'-deoxyuridine-5'-triphosphate (TDBAA-dUTP) - a key compound for enzymatic introduction of ATFMD-groups in DNA fragments suitable for effective DNA/protein crosslinking.

RESULTS AND DISCUSSION

We have prepared dUTP derivative, containing ATFMD-group on the maximal distance about 11-12 Å from heterocycle base, by acylation of 5-aminoallyl-2'-deoxyuridine-5'-triphosphate with 4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoate N-hydroxy-succinimide. The acylating reagent was synthesized starting from 4-bromtoluene according procedure⁷ with modifications⁸. It has been shown that most of the commonly used DNA polymerases - KF of *E.coli* DNA polymerase I, T4 and T7 DNA polymerases, *Taq* DNA polymerase - can utilize TDBAA-dUTP as a substrate in place of natural dTTP.

The TDBAA-dUTP prepared was used for the preparation of 30 bp DNA fragment, containing consensus -10 and -35 prokaryotic promoter elements, with photoactivatable groups being localized in the upper strand of -10 region. This ATFMD-containing DNA fragment can form the specific complex with *E.coli* RNA polymerase like its natural analogue⁹. The UV-irradiation of the complex at 365 nm results in the high-yield covalent DNA-protein crosslinking. Electrophoretic analysis of the reaction products has shown, that all subunits of *E.coli* RNA polymerase, including α subunit, could form covalent linkages with promoter-like ATFMD-duplex.

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