Stereoselective formation of a cyclobutane pyrimidine dimer by using \( N^4 \)-acetyl protection of the cytosine base

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ABSTRACT

The cytosine base in DNA undergoes hydrolytic deamination at a considerable rate when UV radiation induces formation of a cyclobutane pyrimidine dimer (CPD) with an adjacent pyrimidine base. As a part of our study on the synthesis of CPD-containing oligonucleotides, we have prepared properly-protected thymidyl-(3'-5')-\( N^2 \)-acetyl-2'-deoxyctydine, and the solution of this compound was UV-irradiated using acetonaphone as a sensitizer. In this reaction, hydrolysis of the acetylamino group occurred, and a trans-syn cyclobutane thymine-uracil dimer with the syn-anti conformation around the glycosidic bonds was formed stereoselectively.

INTRODUCTION

Among the four nucleobases in DNA, cytosine is the most susceptible to hydrolytic deamination. When UV radiation causes cytosine to form a cyclobutane pyrimidine dimer (CPD) with an adjacent pyrimidine base, the reaction rate of this deamination is greatly accelerated, because stabilization of the amino group by aromatic resonance is lost due to saturation of the 5,6-double bond. The cyclobutane thymine-cytosine dimer (TJ[C]) is converted to the thymine-uracil dimer (TJ[U]) by this deamination. The reported deamination rate constants observed for the cis-syn CPDs of dTpdC and dCpdT at 25°C are 2.5 \( \times \) \( 10^3 \) and 2.8 \( \times \) \( 10^5 \) s\(^{-1} \), respectively. In DNA, various rate constants have been determined, with reported values of 3.9 \( \times \) \( 10^5 \) s\(^{-1} \) at 37°C in vitro and 1.5 \( \times \) \( 10^4 \) s\(^{-1} \) at 42°C in Escherichia coli cells. DNA polymerase \( \eta \), which performs translesion synthesis (TLS), incorporates two adenylates opposite the cis-syn TJ[U], which induces C->T transitions after replication. Therefore, it is of interest to investigate TLS past TJ[C] by pol \( \eta \).

To prevent this hydrolytic deamination during oligonucleotide synthesis, we planned to protect the amino function of the T[C] with a group that can be removed with ammonia in a non-aqueous solution. We prepared a properly-protected thymidyl-(3'-5')-\( N^2 \)-acetyl-2'-deoxyctydine (dTpdacC, 1), and its solution was UV-irradiated with a Pyrex-filtered high-pressure mercury lamp using acetonaphone as a sensitizer.

RESULTS AND DISCUSSION

First, \( N^4 \)-acetyl-3'-O-levulinyl-2'-deoxyctydine was prepared in three steps from \( N^2 \)-acetyl-2'-deoxyctydine following the reported method. After coupling with 5'-O-(4,4'-dimethoxytrityl)thymidine 3'-(2-cyanoethyl)-\( N^2 \)-disopropylphosphoramidite, followed by oxidation with iodine and detritylation with acetic acid, partially-protected dTpdacC (1) was obtained. This product was dissolved in a mixture of acetonitrile and water (1:1 v/v), and the solution was degassed by a nitrogen purge. After acetonaphone was added as a sensitizer, this solution was UV-irradiated with a Pyrex-filtered high-pressure mercury lamp at 5°C for 11 h, and the reaction was monitored by HPLC. Two new peaks showing an absorption spectrum different from that of the starting material emerged. Each product was purified by reversed-phase column chromatography.

At this step, analyses by NMR spectroscopy and high-resolution mass spectrometry revealed that the acetyl group was lost in both products. In other words, a TJ[U] dimer was formed by hydrolysis. To analyze the reaction in detail, the obtained products were subjected to deprotection with aqueous ammonia at room temperature for 2 h, followed by HPLC purification, dialysis and cation exchange.
The deprotected T[J]U was analyzed by NMR. Each proton signal was assigned by COSY. The stereochemistry of the base moiety was determined by truncated NOE experiments. The H-NMR spectra corresponded with that of trans-syn-I T[J]U reported previously,7,8. Besides, the NOE data showed that Tp and pU had syn (an NOE found between H6 and H1') and anti (an NOE found between H6 and H2') conformations around the N-glycosidic bond, respectively. These results indicated that the acetylamino group underwent hydrolysis and that trans-syn-I T[J]U (3) was obtained.

Figure 2A shows HPLC analysis of the deprotected product after UV irradiation of dTpdacC (1). Only a single major peak of trans-syn-I T[J]U (3) was detected. We prepared base-unprotected thymidyl-(3'-5')-2'-deoxyuridine (dTpdC) following the reported method,9 and compared the stereochemistry of the T[J]U obtained after UV-irradiation. The solution of dTpdC was UV-irradiated and deprotected in the same way, and an aqueous solution of the product was kept at 37°C for 24 h to complete the hydrolytic deamination. Figure 2B shows the result of the HPLC analysis of this sample. Three major peaks were detected. According to the previous report10 and photoreversal reaction, they were assigned as cis-syn T[J]U (peak a), trans-syn-I T[J]U (peak b), and trans-syn-II T[J]U (peak c). The observed ratio of the peak areas was a : b : c = 1 : 0.6 : 0.7. In the case of dTpdacC (Figure 2A), this ratio was a : b : c = 1 : 24 : 2.

CONCLUSION
In this study, we analyzed the product after UV irradiation of thymidyl-(3'-5')-N\textsuperscript{6}-acetyl-2'-deoxyuridine. The acetylamino group was hydrolyzed promptly, and trans-syn-I cyclobutane thymine–uracil dimer was obtained exclusively. Although the mechanism remains to be determined, this stereoselective reaction may be used for the efficient synthesis of oligonucleotides containing the trans-syn-I CPD.

REFERENCES

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