Synthesis of Base-substituted dUTP Analogs Carrying a Photoreactive Group and Their Application to Study Human Replication Protein A

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Analogs of dUTP bearing a photoreactivable 2-nitro-5-azidobenzoyl (NAB) group linked with a spacer group containing n-atoms length (n = 2, 4, 7-13) to the 5-position of the uridine ring (NAB-n-dUTP) were synthesized and characterized. These conjugates provide instruments to study nucleic acid - protein interactions. The DNA polymerase β efficiently incorporates these analogs into a radiolabeled primer-template and all analogs substitute for TTP in the reaction of DNA synthesis. That is fruitful way to obtain primer-template duplex carrying one photoreactive group at the very 3'-end of the primer. After completing photoreactive primer synthesis the reaction mixtures were irradiated with monochromatic UV light (315 nm) in the presence or absence of human replication protein A (RPA) and the crosslinking products were separated by SDS-PAGE. The photoreactive primers have been crosslinked with the RPA p70 and RPA p32. Labeling of RPA p14 was not achieved with the photoreactive primers even by varying the length of the spacer group. Analysis of the intensity of primer crosslinking shows that in comparison to the p70 subunit p32 was preferentially labeled by increasing the spacer length. The labeling of pol β is inhibited in the presence of RPA. However, in the case of NAB-n-dUTPs (n = 11, 12, 13) the decrease of pol β modification in the presence of RPA seems to be less efficient.

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