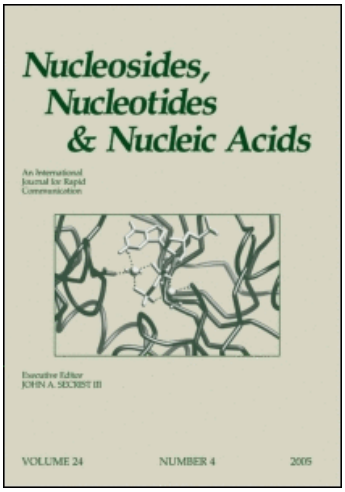

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Photoanalogues of the Initiation Substrates of the RNA Polymerase II, 5-Azido-2-Nitrobenzoyl Derivatives of the ATP γ-Amidophosphate: The Possible Photoinduced Degradation of the Functional Group to an N-Arylhydroxylamine

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ABSTRACT

Photoanlogues of the initiation substrates of the RNA polymerase II, N_3Ar-$NH(CH_2)_nNHpppA$ where N_3Ar is 5-azido-2-nitrobenzoyl group (n=2 or 4) were synthesized, allowing the preparation of photoreactive oligonucleotides *in situ* by RNA polymerase II for application as photolabels. Photolysis of *p*-nitro-substituted aromatic azide in aqueous medium was investigated. Using the azoxy-coupling reaction it was possible to determine whether a nitrene or *p*-nitrophenyl hydroxylamine azoxy compound is the trappable intermediate that is generated at ambient temperature in aqueous solution.

Key Words: Photoanalogues NTP; Aryl azide; Long-lived intermediate.

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INTRODUCTION

Nucleoside 5′-triphosphate derivatives carrying nitro-substituted aryl azide residue were proposed a few years ago as substrates of the nucleic acid polymerases for the preparation of photoreactive oligonucleotides in situ, which can be used for studying supramolecular structures of nucleoprotein complexes.[1,2] Despite this importance, the photochemistry of these compounds in aqueous solution has not been studied in detail. In this paper, we describe substrate activity of ATP photoanalogues 1 and 2 (Scheme 1) in polymerization catalyzed by RNA polymerase II and focus on the photochemistry of its functional group in aqueous media.

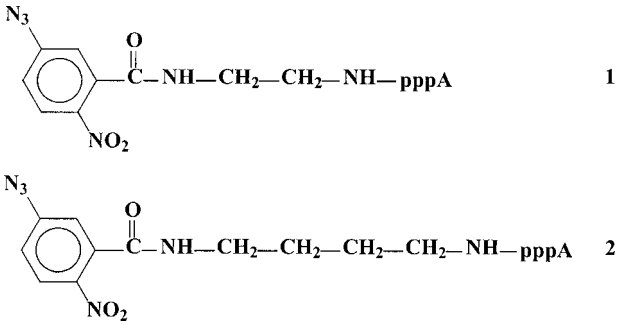
RESULTS AND DISCUSSION

We find that the ATP photoanalogues 1 and 2 have initiation substrate activity similar to those of ATP (Fig. 1, lanes 1 and 2). The fact that synthesis of RNA is aborted (Fig. 1, lane 4) in the presence of α-amanitin (1 μg/ml) demonstrates that transcription is realized by RNA-polymerase II alone.

The photochemistry of *p*-nitro-substituted aromatic azides has been the subject of several previous studies.[3,4] Photolysis of 4-nitrophenyl azide (4NPA) in O_2-saturated organic solution gives *p*-dinitrobenzene, 4-nitronitrosobenzene (4NNB) and 4,4′-dinitroazoxybenzene (4,4′DNAB).[3] The related reaction products have been observed from the photolysis of 4-nitro-substituted aromatic azides in aqueous solution.[4] Also, irradiation of an aqueous deoxygenated solution of this aryl azide gives 4,4′DNAB derivative. The formation of azoxybenzene in the absence of O_2 is an intriguing process. On the contrary, irradiation of 4NPA in deoxygenated organic solution gives azobenzene as the single characterizable product.[3]

In the course of the photolysis giving 4,4′-DNAB, the following three-step process, involving *p*-nitrophenylhydroxylamine (4NPHA) as a transient intermediate, may be adopted on the basis of the facts described below (Scheme 2).

Under the conditions of assays, a primary arylhydroxylamine is susceptible to oxidation, disproportionation and condensation.[5] On the other hand, a related products reaction has been generated from the triplet nitrene.[3] A derivatization scheme was sought that would convert the hydroxylamine to a stable product permitting analysis to be carried out



Scheme 1. Structural formulae of photoreactive ATP derivatives.

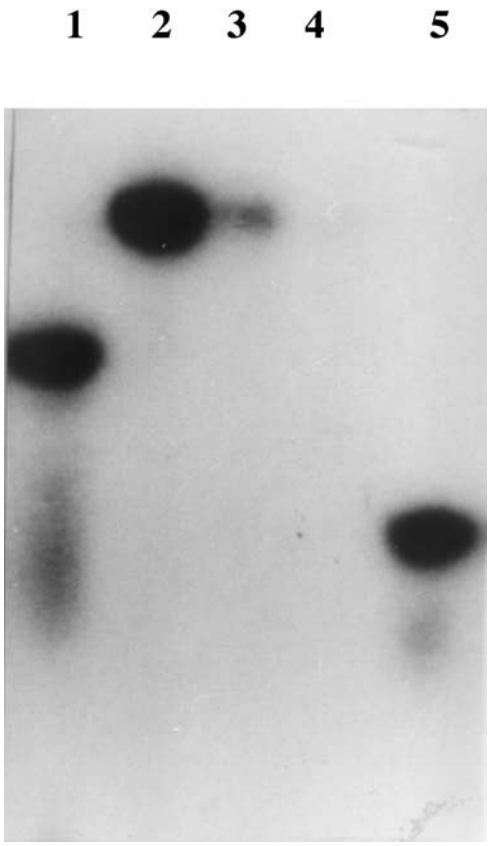
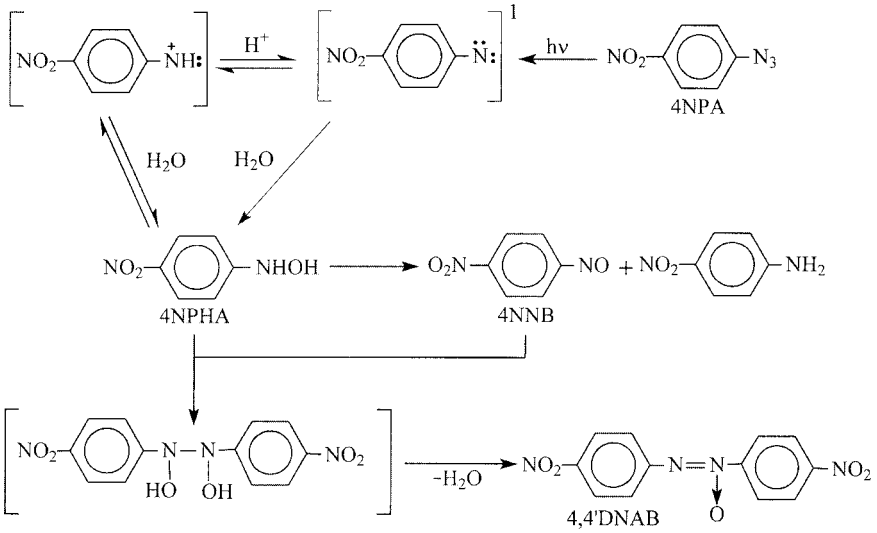


Figure 1. Autoradiogram of electrophoretically analyzed transcripts (20% polyacrylamide gel, 7 M urea): lane 1–transcription with NTP and ATP (4×10^{-4} M); lanes 2 and 3–transcription with NTP and ATP photoanalogue 2 (4×10^{-4} M and 10^{-5} M respectively); lane 4–transcription in the presence of α-amanitin; lane 5–heterologous ^{32}P-labeled 17-base oligoribonucleotide.

at a more leisurely pace with minimal decomposition. The derivatization approach that has been exploited involves the addition of nitroso (as a trap of arylhydroxylamine) to the aryl azide; the photolysis product being converted to a more stable azoxy compound.

We began with an experiment with 4NNB using prephotolyzed 4NPA (0.7/1 mixture) and found that a reaction proceeds to give 4,4′DNAB (68 %). Since it was determined that O_2 has a marked effect on the photochemistry of aryl azides,[3,4] all studies were performed in deoxygenated aqueous solution. Photolysis of 4NPA in the presence of the 4NNB resulted in an increased yield of 4,4′DNAB (Table 1). These observations point to the operation of a route for formation of 4,4′DNAB that does not pass through a triplet nitrene. One possibility for the non-nitrene route to 4,4′DNAB is the reaction of the 4NNB with 4NPHA (Scheme 2).

In photoaffinity labeling experiments, the phenomenon of formation of long-lived species upon irradiation of reagents carrying 5-azido-2-nitrobenzoyl residue has been



Scheme 2. The course of the photolysis of [*p*]-nitrophenylazide aqueous solution.

traced to.[6] Our study has demonstrated that 4NPHA as a reactive intermediate may be formed from photolysis of these photoreagents in aqueous solution.

EXPERIMENTAL PROCEDURES

The plasmid pUC119 containing adenovirus major late promoter (AdML) (−39/+20) was kindly provided by J. Kadonaga. A transcriptionally active complex was prepared from *Saccharomyces cerevisiae* and the transcription reactions were performed as previously described.[7] Our results for the synthesis of the ATP photoanalogues 1 and 2 will be published in due course.

UV irradiation of the samples was performed as previously described.[5] Sample solutions were prepared in quartz cells with Teflon stopcocks and purged with Ar for 20 min at 0°C prior to each experiment. A water solution of 4NNB was mixed with either 5.3×10^{-4} M solution of prephotolyzed 4NPA or with 4NPA (reaction mixture I) in molar ratio according Table 1. The reaction mixture I was subjected to irradiation. All mixtures were cooled in an ice water bath. The resulting precipitates were collected, dried in vacuo and identified as 4,4′DNAB.[8] ^{1}H NMR ($(CD_3)_2CO$, *J*, Hz, δ,

Table 1. Effect of 4NNB on the 4,4′DNAB formed on photolysis of 4NPA.

4NPA/4NNB	Absence of 4NNB	3.6/1	0.7/1
The yield* of 4,4′DNAB, %	15	31	73

*Yields are described as molar equivalent estimated from one mole of the starting 4NPA.

p.p.m.) 8,32 (d.d., $J_{2',3'}=J_{6',5'}=9.0$, $J_{2',6'}=J_{6',2'}=2,5$ H2′, H6′, 2H), 8,44 (d.d., $J_{3',2'}=J_{5',6'}=9.0$, $J_{3',5'}=J_{5',3'}=2,5$ H3′, H5′, 2H), 8,51 (d.d., $J_{3,2}=J_{5,6}=9.0$, $J_{3,5}=J_{5,3}=2,5$ H3, H5, 2H), 8,64 (d.d., $J_{2,3}=J_{6,5}=9.0$, $J_{2,6}=J_{6,2}=2,5$ H2, H6, 2H).

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