Tetrahedron Letters 49 (2008) 5087–5089

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

A thermostable azo-linker for reversible photoregulation of DNA replication

Qi Wang, Long Yi, Liangliang Liu, Chuanzheng Zhou, Zhen Xi *

State Key Laboratory of Elemento-Organic Chemistry and Department of Chemical Biology, Nankai University, Tianjin 300071, China

ARTICLE INFO

Article history:
Received 9 April 2008
Revised 22 May 2008
Accepted 6 June 2008
Available online 12 June 2008

Keywords:
DNA replication
Reversible photoregulation
Thermostable azo-linker
Hairpin DNA

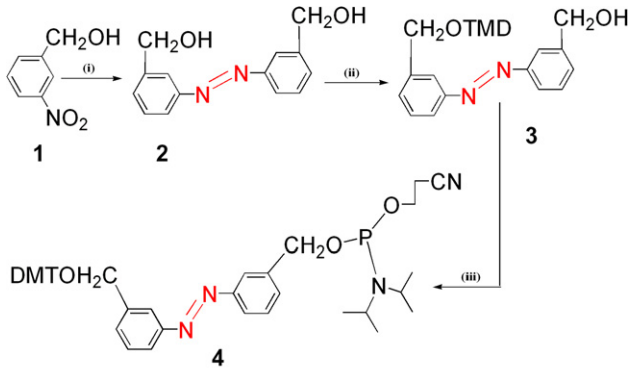
ABSTRACT

Small molecules such as azobenzenes, one of the best reversible photo-switches, can be covalently incorporated into DNA to regulate its structures and functions with irradiation of the specific wavelengths. Using this strategy, a thermostable azobenzene linker was employed to construct modified oligodeoxynucleotides, and we successfully achieve reversible photoregulation of DNA replication in vitro with short irradiation time. Five minutes UV irradiation for regulating trans→cis transformation can minimize DNA damage, still ensure the polymerase reaction of cis-form. Formation of DNA hairpin structure can also be controlled by photoregulation using this linker.

Much research has been centered on modified oligonucleotides, and various types of biological and chemical functionalization have recently been achieved.[1] For example, small molecules such as azobenzenes, one of the best reversible photo-switches, can be covalently incorporated into DNA to regulate its conformation and properties with irradiation of the specific wavelengths.[2] One of the problems caused by the modification of reported azobenzene is degradation of *cis*-azobenzene, which is normally not thermostable enough at 37 °C with $T_{1/2}$ less than 10 h.[3] In fact, introducing an inductive electron-withdrawing group to azobenzene's *meta*-positon, the obtained compound can be an excellent photoswitch.[4] Herein we choose 3,3′-azo-di-benzyl alcohol (**2**) as a photoregulator for two reasons: (I) it can connect two DNA sequences as a linker rather than a side chain fragment, this linker may give attractive properties because it would be in a different proximity imposed by the photoisomerization; (II) it exhibits thermostability for practical use in many biological studies at physiological conditions.

Compound **2** was easily obtained from 3-nitrophenylmethanol,[5] and its photoisomerization efficiency and thermostability are excellent.[4] Compound **2** can be introduced into backbone of DNA using conventional phosphoramidite chemistry (Scheme 1). These modified oligodeoxynucleotides **5–7** contain five, six, and seven bases, respectively, at 3′ terminus following the linker, which can form base pairs with those bases at the other side of the linker.

Compound **2** is an excellent candidate as a photoswitch with good thermal stability of the cis-form.[4] The modified oligodeoxynucleotides containing **2** as a linker were used in DNA polymerase reactions in vitro. Considering the recognizability of KFexo- polymerase,[6] we design and synthesize DNA **5**–**7** including an azobenzene linker for photoinducing cis↔trans isomeriaztion. When the DNA synthesis with KFexo- polymerase was achieved at pH 7.9 and 37 °C in the dark in the presence of oligomer **5**, 5.4% 28-mer DNA was produced, while the same reaction performed under UV irradiation (347 ± 40 nm), nearly no DNA elongation was found. It



5: 5'-dTATCAGACTTAATLATTAA-3'
6: 5'-dTATCAGACATTAATLATTAAT-3'
7: 5'-dTATCAGACTATTAATLATTAATA-3'

Scheme 1. The synthetic scheme of modified azobenzene and the DNA sequences used in this study; L indicates the azobenzene linker derived from **4**. Reagents and conditions: (i) $SnCl_2$, NaOH, H_2O/CH_3CH_2OH, reflux, 85%; (ii) DMT-Cl, pyridine, DMAP, CH_2Cl_2; (iii) diisopropylamine, 1*H*-tetrazole, CH_2Cl_2, 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite, two steps yield, 60%.

* Corresponding author. Tel./fax: +86 22 23504782.
E-mail address: zhenxi@nankai.edu.cn (Z. Xi).

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doi:10.1016/j.tetlet.2008.06.027

should be noted that each linker in the present study nearly equals to 2 nt, and mobility of 28-mer DNA was observed from the gel (Fig. 1, lane 3). The UV irradiation causes no significant effects on the enzymatic activity, as confirmed by careful control experiments. At the same reaction condition, the yields of 30-mer DNA from oligomer **6** with and without UV irradiation are 26.3% and 48.0%, respectively, which imply that UV regulation has significant effect on the reaction in oligomer **6**. It is interesting to note that the UV irradiation causes no significant difference on the reaction of oligomer **7**, as confirmed by the fact that the reaction with and without UV irradiation has 27.5% and 28.6% yields of 32-mer DNA, respectively. These experiments were repeated three times to lead nearly similar results, and the photoregulation of DNA replication depends on the DNA lengths following azobenzene linker at 3′ terminus, namely, high selectivity but low yield for oligomer **5**, both selectivity and high yield for oligomer **6**, and no selectivity for oligomer **7**.

These interesting experiment results imply a possible reaction mechanism as shown in Scheme 2. Considering both the configuration of azobenzene and DNA sequences, DNA chains could form duplex intramolecularly or/and intermolecularly. As a result, the DNA sequences at 3′ terminus of one chain can act as a primer and another chain as a template. While DNA with the cis-form linker can form hairpin structure,[7] the oligonucletides provide both template and primer by itself for DNA replication.

Short base-pairing length of oligodeoxynucleotide (such as in oligomer **5**), giving relatively weak interaction for base-pairing, leads to either the low yield by weak binding from polymerase in dark, or no yield as the inefficacy of polymerase binding to the very short stemmed hairpin structure in the cis-form azobenzene linker under UV for oligomer **5**.

Considering the base-pair numbers and the experimental results, DNA may prefer duplex for the trans-form. While for the cis-form, the hairpin structure in this case can provide both primer and template for DNA polymerase. As a result, both DNA syntheses in the dark and under UV irradiation were observed in oligomer **6**. Note that two DNA with duplex can provide two reaction sites, while there is only one in the hairpin structure. Because the total reaction sites of both cases in the present reaction conditions are the same, the polymerases will diffuse to DNA to give the similar reaction velocity. However, the DNA synthesis yield for the trans-

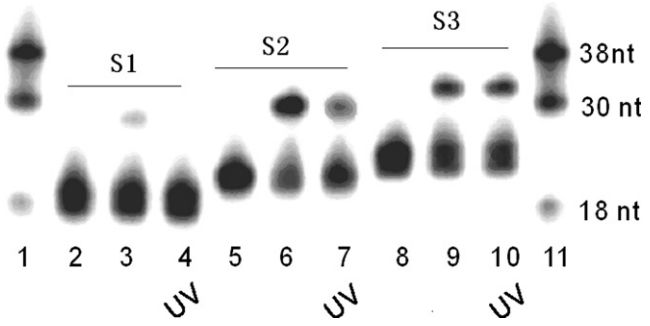
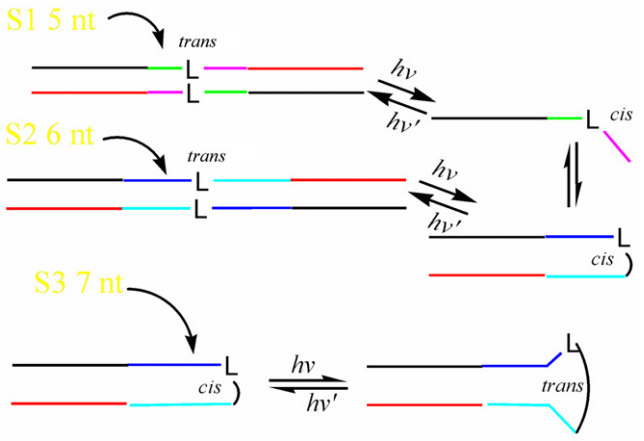


Figure 1. Polyacrylamide gel electrophoresis patterns for Klenow fragment of Escherichia coli DNA polymerase I (KFexo-, 0.02 U/μL) reactions at pH 7.9, 37 °C for 30 min. These oligodeoxynucleotides (30 nM) were 5′-^{32}P-end-labled using [γ-^{32}P] ATP and T4 polyynucleotide kinase. All oligodeoxynucleotides were irradiated with light over 540 nm for 10 min before DNA polymerase reaction to make sure that azobenzene in the oligodeoxynucleotides is of trans-form. The oligodeoxynucleotides used in the reaction under UV irradiation were firstly irradiated by UV light for 5 min, and then added to the reaction tube. Lanes 1 and 11: markers (18-mer, 30-mer, and 38-mer); S1–S3 for **5**–**7**, respectively; lanes 2, 5, and 8: control experiments, reaction without KFexo-; lanes 3, 6, and 9: with modified oligodeoxynucleotides in the dark; lanes 4, 7, and 10: with modified oligodeoxynucleotides under UV irradiation. DNA syntheses were quantitated using Typhoon TRIO Phosphorimager.



Scheme 2. A possible mechanism for DNA polymerase reactions with different lengths of DNA; DNA elongation part, red line; reaction template, black line; short primer, green and purple lines; long primer, blue and cyan lines; base pairs exist between green and purples lines or blue and cyan lines.

form is nearly two times (1.83:1) higher than that for the cis-form. We suppose that if one polymerase attaches one reaction site of the trans-form, it can very quickly transfer to another reaction site of this DNA by a 'supra-intramolecular jumping' rather than the normal diffusion from polymerase to DNA, which leads to expedite the reaction in this case.

Increasing the DNA length as in oligomer **7** gives a better hairpin structure for both *cis*- and *trans*-azobenzene linker, due to entropy effect of intramolecular base-pairing interactions that overcome the stereo effect of azobenzene even in the trans-form. So the DNA synthesis yields with and without UV irradiation are similar in oligomer **7**, which is also the case as in the polymerase reaction of TATCAGACATATTAATLATTAATAT (data not show).

Oligomer **6** was further chosen for the investigation of reversible photoregulation (Fig. 2). The DNA syntheses in the dark and under UV irradiation for 1 h give similar results as that found in Figure 1, and the yields ratio is nearly 2:1. Half an hour reaction under UV irradiation followed by half an hour reaction under visible light gave 27.1% yield. One hour reaction under UV irradiation gave 16.2% yield, while that in dark 31.3%. These results imply that after half an hour UV irradiation, the followed visible light irradiation makes cis→trans transformation of the linker that causes opening of the hairpin structure (Scheme 3).

In practical application of photoregulation, the UV irradiation time for artificial regulation on DNA replication should be as short as possible to avoid possible DNA damage. In this work, a thermostable azobenzene linker was designed to overcome this shortcom-

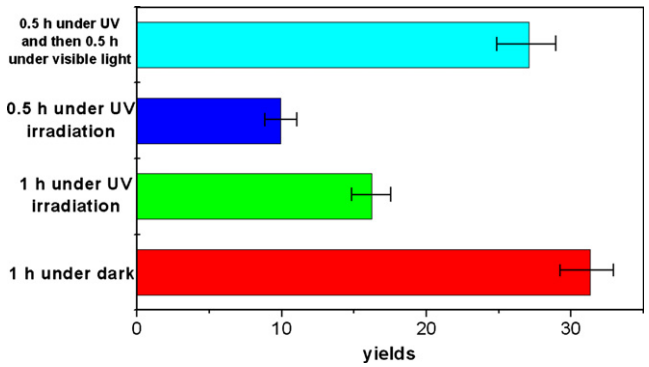
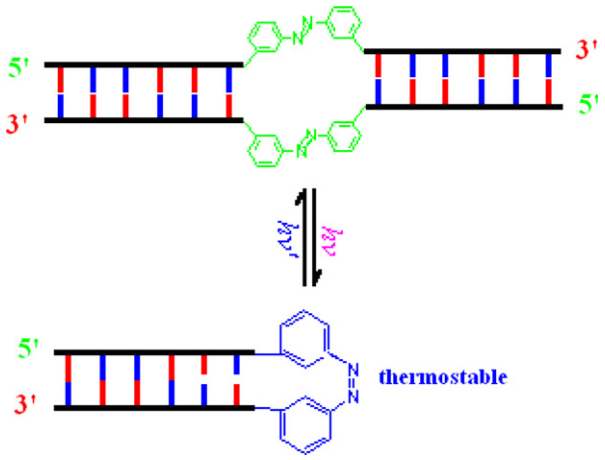


Figure 2. The reaction yields of **6** irradiated by a different light (see Fig. S3 for the gel patterns).



Scheme 3. Reversible photoregulation of DNA hairpin and duplex structure by UV/visible light; blue, T; red, A.

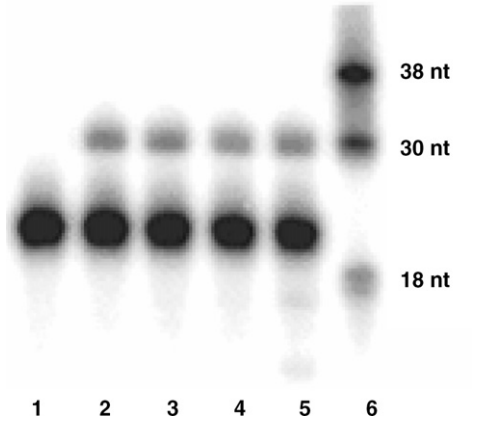


Figure 3. Polyacrylamide gel electrophoresis patterns for DNA reactions of **6** with similar conditions of the reaction in Fig. S3; **6** was firstly irradiated by UV irradiation for 5 min and then added to the reaction tube. Lane 1, control; lanes 2–5, performed the reaction at 0, 5, 25, 55 min under UV irradiation, respectively, and then in the dark (the total reaction time for each case is 55 min); lane 6, marker.

ing. Five minutes UV irradiation time is enough to keep DNA replication going for the cis-form azobenzene type reaction due to the significantly increased thermostablility of the designed *cis*-azobenzene linker **2** ($T_{1/2}$ = 45.6 h at 37 °C). No need for uninterrupted irradiation to secure cis-form reaction, the reaction yield is nearly same for different irradiation time as shown in Figure 3. Note that extra fast moving bands were slightly visible after 1 h UV irradiation, indicating possible DNA radiation damage for long time UV irradiation (Fig. 3, lane 5). Five minutes UV irradiation can minimize DNA damage, still ensuring the polymerase reaction of cis-form.

In conclusion, the results reported here indicate that reversible photoregulation for the DNA replication can be achieved through incorporating a thermostable azobenzene linker. Formation of DNA hairpin structure can also be controlled by photoregulation using this linker. We are exploring the potential applications of this thermostable linker to regulate different DNA/RNA functions.

Acknowledgments

This work was supported by the National Key Project for Basic Research of China (2003CB114403), National Natural Science Foundation of China (20272029, 20572053, 20421202, 20432010), Ministry of Education of China (104189, B06005) and Nankai University ISC.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.06.027.

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