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Photochemical Halogen-Exchange Reaction of 5-Iodouracil-Containing Oligonucleotides

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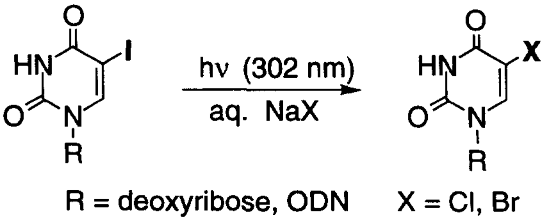
Abstract: Photoreactions of 5-iododeoxyuridine (d^{I}U) and d^{I}U-containing oligonucleotides in aqueous solutions in the presence of various inorganic salts have been investigated. In the presence of NaCl and NaBr, d^{I}U and d^{I}U-containing oligonucleotides undergo an efficient photochemical halogen-exchange reaction to give d^{Cl}U and d^{Br}U, respectively. © 1999 Elsevier Science Ltd. All rights reserved.

Photoreactions of 5-halouracils are important tools for investigating nucleic acid structures and nucleic acid-protein interactions.[1] Especially, 5-iodouridine (d^{I}U) has been widely used as a photo-cross-linking agent, and the photo-cross-linking of d^{I}U-containing DNA with nucleic acid binding proteins has been extensively studied.[2] The UV-irradiated d^{I}U-containing DNA has also been known to cause DNA strand breaks via the creation of alkali labile sites, which also explains the enhanced photosensitivity of the cell containing d^{I}U-substituted DNA.[3] We have been investigating the molecular mechanism of such DNA lesions and the effect of a DNA conformational change on the photoreactivity of d^{I}U- and d^{Br}U-containing DNAs.[4] During careful product analysis of the photoreaction of d^{I}U-containing-oligonucleotides, we accidentally found the formation of 5-chlorouridine (d^{Cl}U) from d^{I}U, which resulted from the halogen exchange with NaCl used in an aqueous buffer solution. While many inorganic salts exist in a biological system, direct photoreactions of 5-halouracil-containing DNA or RNA with inorganic salts have not been recognized so far. We investigated the photoreactions of d^{I}U in the presence of various inorganic salts and found that the 5-iodo substituent of d^{I}U is efficiently replaced by other halogen atoms. We also applied this novel photo-halogen-exchange reaction to the post-modification of d^{I}U-containing DNA.



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The photoirradiation of monomer d^{I}U with a transilluminator (302 nm) in water in the presence of 100 mM 2-propanol as H-donor gave deoxyuridine (dU) as the sole product. Upon photoirradiation of d^{I}U in the presence of 4 M NaCl, d^{Cl}U was produced in high yield in addition to the formation of dU. Similarly, when the photoirradiation of d^{I}U was conducted in the presence of 4 M NaBr, the halogen exchanged product, d^{Br}U, was also obtained as a major photoproduct together with dU as shown in Figure 1 and Table 1. In both cases, the consumption of d^{I}U was remarkably enhanced by the addition of salts. However, the photochemical exchange of d^{I}U with fluoride, which gives the medicinally important 5-fluorodeoxyuridine, was not successful. To verify the effect of halogen anions, the photoreaction in the presence of NaI was compared with that in the presence of $NaClO_4$. It was found that the added NaI significantly accelerated the consumption of d^{I}U, whereas $NaClO_4$ had a rather inhibitory effect on the disappearance rates of d^{I}U. These results indicated that the presence of a halogen anion rather than the ionic strength is responsible for the accelaration of the photoconversion of d^{I}U, whereas $NaClO_4$ had rather ihibitory effect on the diappearance of d^{I}U, probably due to the ionic relaxation.[5]

Table 1. Effect of Inorganic Salts on the Photoreaction of d^{I}U[a]

		Consumption (%)	Products (%)		
Run	Salt	(-d^{I}U)	dU	d^{Cl}U	d^{Br}U
1	no salt	29	29	-	-
2	NaCl 4 M	49	30	19	-
3	NaBr 4 M	87	39	-	47
4	NaI 4 M	61	61	-	-
5	$NaClO_4$ 2 M	17	13	-	-

[a] Each of the reaction mixtures (200 μL) containing 100 μM d^{I}U, 100 mM *i*-PrOH and inorganic salts in a capillary cell was irradiated for 10 min at 0 °C with a transilluminator (302 nm). The reaction mixture was directly analyzed by HPLC on a CHEMCOBOND 5-ODS-H column (4.6 x 150 mm), detected at 280 nm. Elution was with 0.05 M ammonium formate containing 2 - 10 % acetonitrile linear gradient (20 min), at a flow rate of 1.0 mL/min, at 30 °C.

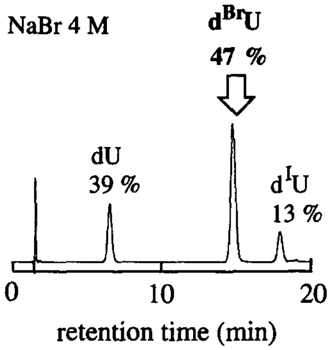


Figure 1. HPLC profiles for UV-irradiated d^{I}U in the presence of 4 M NaBr.

We next examined the effect of a halogen anion on the photoreaction of the d^{I}U-containing oligonucleotides. Likewise, the d^{I}U-containing oligonucleotides undergo an efficient photo-halogen-exchange reaction with NaCl and NaBr to yield the d^{Cl}U- (**1**, **4**) and d^{Br}U-containing oligonucleotides (**2**, **5**), respectively, along with 2'-deoxyribonolactones (**3** and **6**)[4] as minor side products as shown in Scheme 1 and Table 2.[6] These results clearly show that d^{Cl}U- and d^{Br}U-containing DNAs are readily accessible by photochemical post-modification of the d^{I}U-containing DNA in the presence of appropriate inorganic salts. It is noteworthy that the d^{Br}U-containing oligonucleotide also undergoes a photo-exchange reaction in the presence of NaI to provide the d^{I}U-containing oligonucleotide (run 7). These results suggest that this photo-halogen-exchange reaction may also be very useful for the preparation of isotopically labeled d^{I}U-containing DNA using Na^{125}I.

Scheme 1

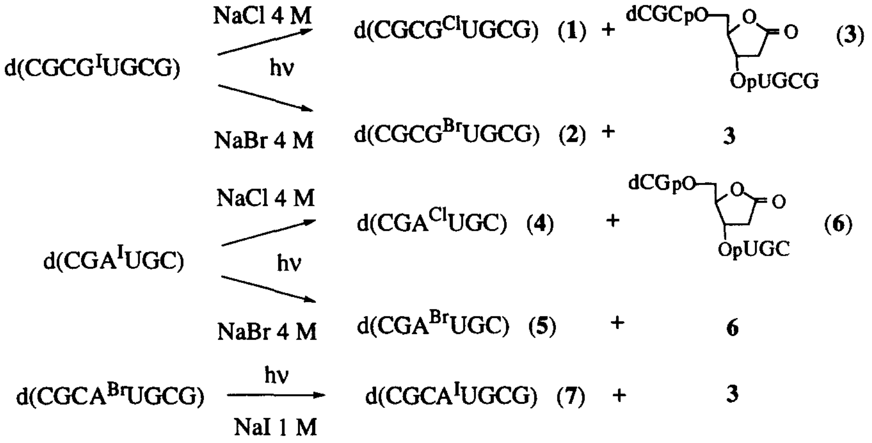


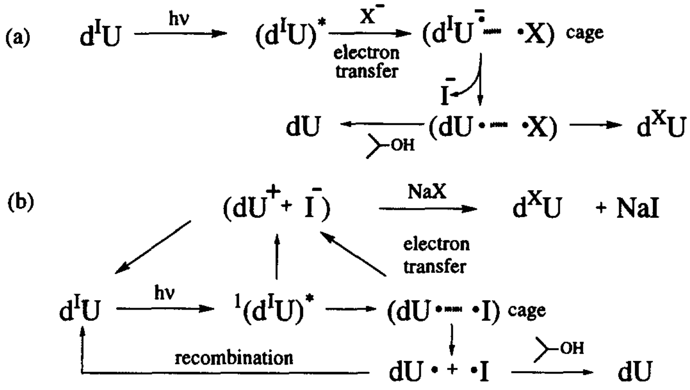
Table 2. Product Analysis in Photoirradiation of 5-Halouracil-Containing Deoxyoligonucleotides[a]

ODN	Run	Salt	Consumed ODN, %	Halogen exchanged product (%)	Ribonolactone (%)
d(CGCG^{I}UGCG)	1	no salt	10	-	**3** (3>)
	2	NaCl 4 M	51	**1** (20)	**3** (3>)
	3	NaBr 4 M	93	**2** (46)	**3** (3>)
d(CGA^{I}UGC)	4	no salt	58	-	**6** (13)
	5	NaCl 4 M	77	**4** (22)	**6** (9)
	6	NaBr 4 M	99	**5** (48)	**6** (7)
d(CGCA^{Br}UGCG)[b]	7	NaI 1 M	70	**7** (15)	**3** (23)

[a] Each of the reaction mixtures (100 μL) containing ODN (0.20 mM base conc.) in 50 mM sodium cacodylate buffer (pH 7.0) in a capillary cell was purged with argon for 2 min and irradiated for 1 h at 0 °C with transilluminator (302 nm) under the described salt conditions. [b] Photoirradiation was conducted for 10 minutes.

Rappoport *et al.*[7] have reported the photoinduced $S_{RN}1$ reaction of vinyl halides. Therefore, one plausible mechanism for the present photo-halogen-exchange reaction may be such $S_{RN}1$ route, which takes place through the intermediacy of the vinyl radical as shown in Scheme 2a. Previous studies on the photoreactions of vinyl iodides have indicated that in organic solvents, vinyl iodides can afford photoproducts which are derived from carbocationic intermediates. Such carbocationic intermediates have been suggested to arise from an electron transfer within an initially formed caged radical pair.[8] Therefore, another possible mechanism of the present photo-halogen-exchange reaction may involve nucleophilic attack of the halogen anion (X^-) on the carbocation generated at uracil C5 as shown in Scheme 2b. However, such a carbocationic intermediate has hitherto not been experimentally detected in the previous photoreactions of d^{I}U.[2] When the photoirradiation of d^{I}U was conducted in the presence of several nucleophiles such as methanol and ethane-1,2-diol,[8a] there were no indications of the formation of the products derived from the nucleophilic trapping of the carbocationic intermediate dU$^+$. Further study is apparently necessary for the elucidation of the mechanism of this novel photoreaction.

Scheme 2



In summary, we have demonstrated for the first time that d^IU- and d^IU-containing DNAs undergo an efficient photo-halogen-exchange reaction in the presence of halide ions to produce C-5 halogen exchanged uracils. This efficient photoreaction of 5-halouracils in the presence of inorganic salts should be seriously taken into consideration when photo-cross-linking experiments using d^IU- or $d^{Br}U$-containing DNA are conducted in high salt-containing solvent systems.

References and Notes

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6. The structures of products **1**-**7** were confirmed by enzymatic digestion to nucleoside and by electrospray ionization mass spectroscopy (ESMS). ESMS (negative); **1**: calcd 2447.1, found 2446.2. **2**: calcd 2491.5, found2491.4. **3**:calcd2277.5, found2277.1. **4**:calcd1812.7, found1811.8. **5**:calcd1857.1, found 1855.8. **6**: calcd 1659.1, found 1658.8. **7**: calcd 2522.5, found 2521.7.
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