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Production of radical species and modification of DNA through one-electron reduction with indium metal

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Abstract—Indium metal produced radical species on DNA through one-electron reduction of 5-iodouracil, and DNA modification via a radical intermediate became possible by the use of an aqueous mixture containing both indium and a radical acceptor.

Radical generation is one of the significant events in organisms; in particular, radical-induced damage to DNA plays a crucial role in carcinogenesis via the formation of abnormal base pairs.[1–4] Therefore, the control of site-specific radical production in DNA is expected to contribute to the further understanding of the effect of radical species on biomolecules and to make it possible to site-selectively incorporate functional molecules into biomolecules via a radical reaction.

Photolysis of 5-halouracils by UV irradiation is well-known as a technique for effective radical generation on DNA.[5–7] Hydrogen atom abstraction from DNA by reactive oxygen species, such as a hydroxyl radical and a peroxyl radical, is also a common radical-generating reaction.[8–11] However, several reagents and stimulations are simultaneously required for the activation, and sometimes they cause undesired side reactions. In addition, regulation of the reaction sites or reactivity of radical species is not easy. Thus, development of a radical generation method available under mild aqueous conditions is strongly desired for biomolecules.

For this purpose, we focused on indium metal because indium has approximately the same first ionization potential as alkaline metals and acts as an effective electron donor; the first ionization potentials are 5.79 eV for indium, 5.12 eV for sodium, and 5.39 eV for lithium.[12,13] It is noteworthy that indium shows a much higher stability in water than lithium and sodium, and efficiently provides one electron to electron acceptors in aqueous media. Several significant radical reactions with indium in aqueous media, such as Barbier-like allylation,[14–16] dehalogenation,[17] and reduction of nitro and azide groups,[18–20] have been reported previously. Therefore, this unique metal property would be effective and useful for the generation of radical species in DNA.

Herein, we report site-specific radical generation in DNA using dehalogenation of 5-halouracils with indium metal. Halouracils were reduced using sonication in the presence of indium metal in water. The indium reduction of 5-iodouracil-containing DNA was applied to a variety of site-specific modifications via the formation of a radical intermediate in aqueous media.

We prepared four 5-halouracil bases: 5-fluorouracil; 5-chlorouracil; 5-bromouracil; and 5-iodouracil; to investigate their reactivity in the presence of indium(0) in aqueous solutions. An excess amount of indium powder (100 μmol) was added to a solution of 5-halouracil (1 μmol) in water, and the resulting suspension was incubated with sonication at room temperature. Consumption of 5-halouracil was monitored by HPLC (Fig. 1). Consumption of 5-fluorouracil and 5-chlorouracil was negligible in 24 h, showing that they have very low affinity for indium metal. In contrast, 5-bromouracil was consumed with $t_{1/2} = 7.5$ h in the presence of indium metal. In the HPLC profile, a new peak appeared with a decrease in the peak due to 5-bromouracil (Fig. 2).

Keywords: Indium; DNA; Radical; One-electron reduction; 5-Halouracil.
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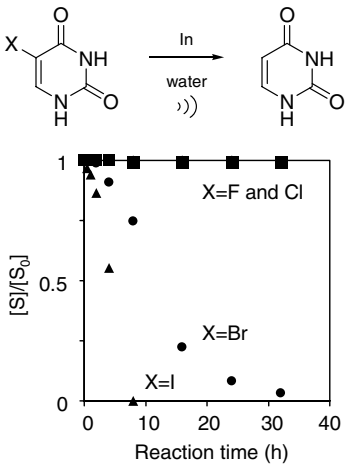


Figure 1. Time course of consumption of 5-halouracils in the presence of indium metal. The reactions were monitored with HPLC at 260 nm as shown in Figure 2. The reaction solution containing 5-halouracils and indium powder was sonicated at 25 °C for the indicated time.

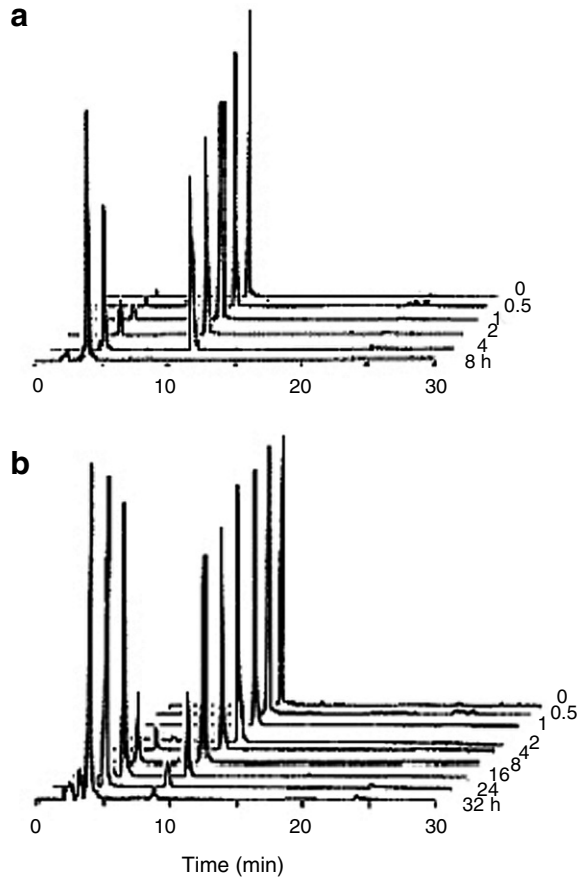


Figure 2. HPLC monitoring of the indium(0) reduction of (a) 5-iodouracil and (b) 5-bromouracil. The reaction samples were eluted with a solvent mixture of 100 mM triethylammonium acetate, 5–20% acetonitrile for 30 min at a flow rate of 1.0 mL/min. The new peak at 4 min was identified as a uracil.

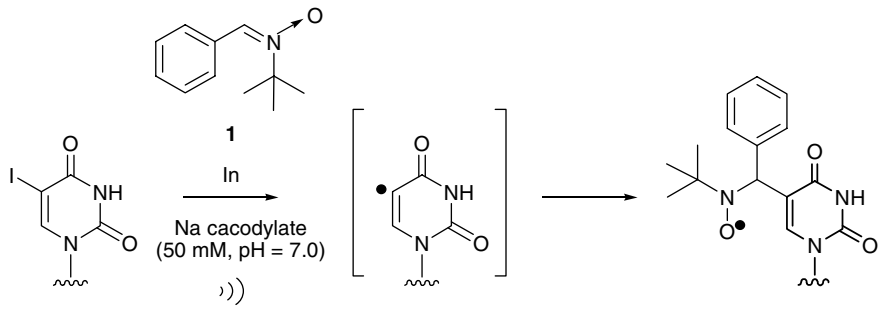
The new product, collected and analyzed by ESI mass spectrometry, was identified as a uracil base ($[M+Na]^+$, calcd 135.0; found 135.2), showing that dehalogenation via one-electron reduction occurred in the aqueous mixture.[21] Additionally, 5-iodouracil was more rapidly converted into uracil ($t_{1/2} = 4.5$ h). Consumption of the five natural DNA/RNA nucleobases—guanine, adenine, cytosine, thymine, and uracil—was not observed under the same reaction conditions. Sonication was used in these reactions, because sonication is effective for activation of indium surface. Consumption of 5-halouracil was not observed when a reaction sample was incubated without sonication.

Having established the reactivity with nucleobases, we next examined the reactivity of indium with an oligodeoxyribonucleotide (ODN) strand. A 5-iodouracil-containing ODN 5′-d(GCA^{I}UGC)-3′ was prepared,[22,23] and the reaction solution containing 5′-d(GCA^{I}UGC)-3′ (50 nmol) and indium powder (50 μmol) in 50 mM sodium cacodylate (pH 7.0) was incubated with sonication at 25 °C for 12 h. A single product was obtained and assigned as a reduced ODN 5′-d(GCAUGC)-3′ using MALDI-TOF mass spectrometry ($[M-H]^-$, calcd 1777.18, found 1777.16). The mass of the product indicates that the ODN was reduced site specifically and other natural nucleotides in the ODN were not damaged under the indium-containing conditions.[24,25]

The indium reduction of DNA can be applied to a variety of modifications of DNA. In order to modify the radical generation site in DNA, we examined a reaction in the presence of a spin trap, *N*-*tert*-butyl-α-phenylnitrone **1** (500 nmol). As a result, **1**-adduct as well as the reduced product were produced (13% yield, $[M-H]^-$, calcd 1953.40, found 1954.08) (Scheme 1). The formation of **1**-adduct proves that a radical is generated on DNA, and indicates that DNA modification via a radical intermediate with a short life time is possible in aqueous media by the use of a mixture containing both indium and a radical acceptor.

We also applied the radical formation reaction with indium(0) and 5-iodouracil in DNA to the initiation step in the radical polymerization of acrylamide. The ODN 5′-d(GCA^{I}UGC)-3′ (50 mM)[26] was incubated with sonication in the presence of indium powder (200 μmol) in a solution containing 40% acrylamide, 0.8 M methylene bisacrylamide, and 5 μM *N*,*N*,*N*′,*N*′-tetramethylethylenediamine at 25 °C for 12 h. The formation of a white gel was observed when both 5′-d(GCA^{I}UGC)-3′ and indium metal were added to the sample solution (Fig. 3). In contrast, there was no gelation of the sample solution in the absence of either indium metal or 5′-d(GCA^{I}UGC)-3′. This is a unique polymerization triggered by DNA and metal, which may be applicable to assaying for a DNA strand containing 5-iodouracil as a surrogate of thymine.

In conclusion, indium metal acted as an efficient reductant for 5-halouracils in aqueous media, and site-specific radical formation in DNA was achieved with 5-iodouracil and indium without other DNA damage. Radical incorporation into DNA using indium was applied to a variety of modifications to DNA through reactions with radical acceptors. The one-electron reduction of DNA with indium metal can be induced under mild con-



Scheme 1.

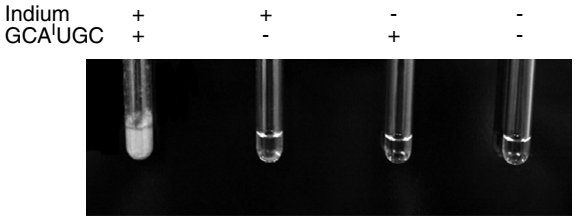


Figure 3. Polymerization of acrylamide initiated by radical formation on an iodouracil-containing DNA. The reaction solution was incubated for 12 h at room temperature after sonication for 3 h.

ditions in aqueous media, and thus indium reduction is a valuable technique to exploit new modification methods for biomolecules.

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21. The hydrogen donor in these reactions is still unknown. Further work on the mechanistic aspects is in progress in our laboratory.
22. Iodouracil-containing ODN 5′-d(GCA^{I}UGC)-3′ was synthesized by a conventional phosphoramidite method using an Applied Biosystems 392 DNA/RNA synthesizer. Synthesized ODN was purified by reversed phase HPLC on a 5-ODS-H column (10×150 mm, eluted with a solvent mixture of 0.1 M triethylammonium acetate, pH 7.0, linear gradient over 30 min from 5% to 20% acetonitrile at a flow rate 3.0 mL/min). Mass spectra of ODN purified by HPLC was measured with MALDI-TOF mass spectrometry with 2′,3′,4′-trihydroxyacetophenone (THAP) as a matrix, using T_8 ($[M-H]^-$ 2370.61) and THAP ($[M-H]^-$ 167.1388) as internal standards. MALDI-TOF MS for 5′-d(GCA^{I}UGC)-3′: found 1902.99 (calcd for $[M-H]^-$ 1903.07).
23. The T_m of this self-complementary ODN was 20 °C. The reactions were examined for the single-stranded state of the ODN.
24. Degradation of the ODN was not observed on incubation with sonication in the absence of indium powder.
25. The product of intramolecular hydrogen abstraction from the 2′-deoxyriboses of flanking nucleotides, that is, C1′ and C2′ hydrogen abstractions, was not observed. Approach of the indium metal surface to DNA may cause the DNA structure to change and prevent hydrogen abstraction, which was observed for B-DNA.
26. A high concentration of ODN was used to observe the appearance of a milky turbidity by the formation of polymer.