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Pyrenylboronic Acid as a Novel Entry for Photochemical DNA Cleavage

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Abstract: 1-Pyrenylboronic acid is bound to supercoiled double strand circular DNA plasmid ColE1 and effects its efficient photocleavage under visible light irradiation: the cleavage reaction, which occurs according to the radical mechanism, can be suppressed by the addition of D-fructose because of boronic acid-saccharide complexation.
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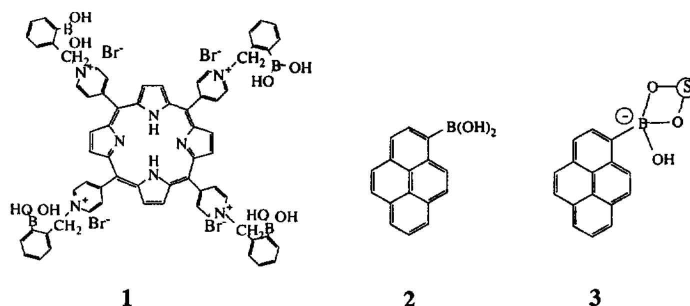
The development of boronic acid receptors for saccharides has recently gained much attention.[1-6] It has been shown that this interaction is superior to the hydrogen-bonding interaction for sugar sensing in an aqueous system.[1-6] One major change induced in the boronic acid by the cyclic ester formation with saccharides is a shift of the pK_a to lower pH region.[1] Hence, one can convert neutral boronic acid $RB(OH)_2$ to anionic boronate ester $R(OH)B^{-}<^{O}_{O}>Sac$ ($Sac<^{OH}_{OH}$: saccharide) at constant pH simply by the addition of saccharides. This phenomenon was successfully applied to the saccharide control of the binding ability of boronic-acid-appended DNA intercalators such as **1**.[7] Here, we came across an interesting reference which describes that the C-B bond in arylboronic acids is susceptible to photochemical cleavage.[8] It thus occurred to us that 1-pyrenylboronic acid (**2**)[9] would also act as a novel photochemical DNA cleavage reagent because (i) pyrene derivatives act as an intercalator,[10,11] (ii) they can be photochemically excited at the wavelength longer than the absorption band of DNA[12], and (iii) the photochemical C-B bond fission should provide some species active for DNA cleavage. We here report on the preliminary results that **2** is bound to DNA under the physiological conditions and photochemically cleaves DNA according to the radical mechanism.

A spectral change in **2** induced by the addition of calf thymus DNA is shown in Fig. 1. It is seen from Fig. 1 that with increasing DNA concentration the absorption maxima at 326 nm and 342 nm decrease while a new absorption maximum appears at 350 nm. This spectral change suggests that **2** is bound to calf thymus DNA mainly due to the π-π stacking interaction. Although a shorter wavelength isosbestic point at around 317 nm is disordered by the background absorption of calf thymus DNA, a longer wavelength isosbestic point at 347 nm is very tight. This suggests that the **2** binding to calf thymus DNA occurs according to a simple one-step process. The binding constant (K) and the mole number of **2** bound to per mole of base pairs (n) were estimated from the Scatchard plot[13,14] (correlation coefficient 0.98): $K=1.27\times10^5$ dm^3 mol^{-1} and $n=0.008$. These values are comparable with those determined for pyrene: $K=(1.6\sim5.1)\times10^4$ dm^3 mol^{-1} and $n=0.005\sim0.015$.[10,11] The similar experiment was carried out for ColE1: $K=4.0\times10^5$ dm^3 mol^{-1} and $n=0.025$.



On the other hand, a spectral change in **2** was scarcely induced in the presence of D-fructose (0.10 mol dm^{-3}). This implies that anionic boronate ester **3** formed from neutral **2** and D-fructose is scarcely bound either to calf thymus DNA or to ColE1.

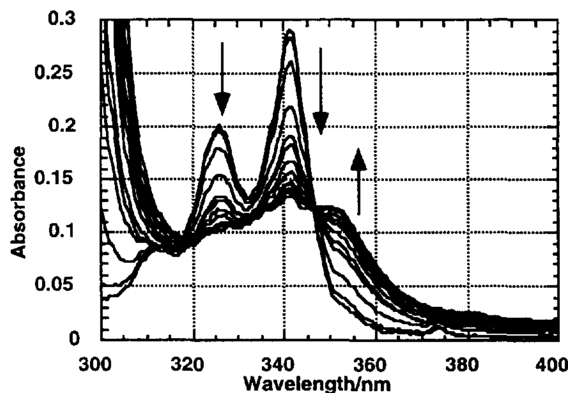


Fig. 1. Absorption spectral change in **2** (1.0×10^{-5} mol dm^{-3}) induced by the addition of calf thymus DNA (2.5×10^{-4} ~3.0×10^{-3} mol dm^{-3}): 25°C, water-methanol=300:1 v/v, pH 7.50 with 10.0 mmol dm^{-3} hydrogencarbonate buffer.

Photochemical cleavage of **2** (1.00×10^{-5} mol dm^{-3}) was carried out at 30 °C in H_2O/CH_3CN (3:7 v/v). A 365 nm longwave UV tube transilluminator (Ultra Violet Products Limited, LMS-20E) was used as a light source.[15] After 30 min the solution was subjected to HPLC analysis (J'sphere ODS-H80, H_2O/CH_3CN(3:7 v/v), 30 °C). The analytical result showed that pyrene is yielded in 76.2% yield. When the photochemical reaction was carried out in D_2O/CH_3CN or H_2O/CD_3CN, the recovered pyrene contained 8.8% or 7.5% pyrene-d, respectively (analyzed by EI-MS). The results show that the hydrogen abstraction occurs rather nonselectively. The photochemical reaction of **2** was also carried out in aqueous solution (30 °C, pH 7.50 with 10.0 mmol dm^{-3} hydrogencarbonate, 0.1 vol% CH_3OH) in the presence of calf thymus DNA (3.3×10^{-5} mol dm^{-3}). After 30 min photoirradiation the absorption spectrum became identical to that of calf thymus DNA plus pyrene. One can consider, therefore, that the same elimination reaction takes place in the presence of calf thymus DNA.

The DNA photocleavage experiments were performed by illumination of an aqueous solution (pH 7.50 with 10.0 mmol dm^{-3} hydrogencarbonate) containing double strand supercoiled circular DNA plasmid ColE1 (cDNA 1.8×10^{-6} mol dm^{-3}) and **2** (3.8×10^{-6} mol dm^{-3}) for 30 min with the transilluminator at 30 °C from a 10 cm distance (4900 $\mu W/cm^2$).[15] The cleavage products were analyzed by gel electrophoresis, monitoring the conversion of supercoiled cDNA (form I) into nicked cDNA (form II) and into linear DNA (form III). The typical experimental results are shown in Fig. 2.

It is seen from lanes A1 and A2 that restriction enzyme *Eco*RI perfectly converts form I into form III. Comparison of lane A3 with lane A4 indicates that compound **2** is inactive in the dark whereas it can convert form I into form II under photoirradiation: that is, **2** efficiently cleaves cDNA with the aid of light. As a control experiment, we used pyrene instead of **2** and tried the photocleavage in a same manner as lane A4. The gel

electrophoresis showed that cDNA is not cleaved at all as shown in lane B3. On the other hand, the addition of D-fructose, which changes a neutral boronic acid to an anionic boronate ester, efficiently suppresses the cleavage activity (lane A5). The tendency is in line with the spectroscopic data that the addition of D-fructose induces the dissociation of **2** from cDNA. This is further supported by the fact that the addition of the same amount of isopropyl alcohol (used as a non-boronic-acid-binding reference compound) does not affect the cleavage activity at all. These findings establish that compound **2** serves as a novel cDNA cleavage reagent, the activity of which is controllable by two dimensional factors, *i.e.*, light and saccharides.

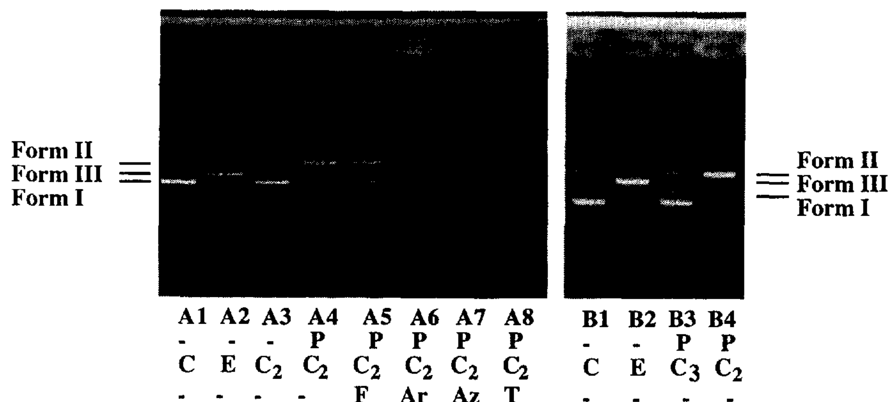


Fig. 2 Gel electrophoresis showing results of photochemical cleavage of supercoiled circular double strand DNA plasmid ColE1 (form I) into nicked cDNA (form II) and into linear DNA (form III): lane A1 and B1, control (C: untreated cDNA 1.8×10^{-6} mol dm^{-3}); lane A2 and B2, cDNA with restriction enzyme EcoRI (E: 0.1 unit/μl); lane A3, cDNA plus **2** (C_2: 3.8×10^{-6} mol dm^{-3}) in the dark; lane A4 and B4, cDNA plus **2** under photoirradiation (P); lane A5, cDNA plus **2** under P in the presence of D-fructose (F: 1.00×10^{-2} mol dm^{-3}); lane A6, cDNA plus **2** under P under anaerobic (Ar) conditions; lane A7, cDNA plus **2** under P in the presence of NaN3 (Az:1.00×10^{-3} mol dm^{-3}); laneA 8, cDNA plus **2** under P in the presence of tetramethylpiperidinyloxy radical (T:1.00×10^{-3} mol dm^{-3}); lane B3, cDNA plus pyrene (**C_3:** 3.8×10^{-6} mol dm^{-3}) under P.

In general, the photochemical DNA cleavage mechanisms are classified into two categories; that is, photochemical generation of $^1O_2$[16,17] or radical species.[15,18] As shown in lane A6, the cleavage activity of **2** is further enhanced under anaerobic (Ar) conditions and cDNA is partly cleaved to give form III. The result supports the view that the activation of O_2 is not essential in the present system. This proposal is further corroborated by lane A7 which shows that the addition of NaN_3 (as a $^1O_2$ quencher) is totally ineffective. In contrast, when tetramethylpiperidinyloxy radical (TEMPO) as a radical scavenger was added, the cleavage activity was reduced to 41~59% (repeated three times: lane A8). In a separate study, we have confirmed from fluorescence spectroscopy that TEMPO dose not quench the excited state of **2**. These results now allow us to firmly propose that the C-B bond in **2** is photochemically cleaved to generate a radical species, which eventually cleaves cDNA.

In conclusion, **2** (and more in general, arylboronic acids) is useful as a potential photochemical DNA cleavage reagent. Further studies are currently continued in these laboratories.

References

1. For comprehensives reviews see, James, T. D.; Sandanayake K. R. A. S.; S. Shinkai, *Supramol. Chem.*, **1995**, *6*, 141-157; James, T. D. ; Linnane P.; S. Shinkai, *J. Chem. Soc., Chem. Commun.*, **1996**, 281-288.
2. Mohler, L. H.; Czarnik, A. W., *J. Am. Chem. Soc.*, **1993**, *115*, 2998-2999and references cited therein.
3. Westmark, P. R.; Smith, B. D., *J. Am. Chem. Soc.*, **1994**, *116*, 9343-9344.
4. Wulff, G.; Heide, B.; Helfmeier, G., *J. Am. Chem. Soc.*, **1986**, *108*, 1089-1091 and references cited therein.
5. Nagai, Y.; Kobayashi, K.; Toi, H.; Aoyama, Y., *Bull. Chem. Soc. Jpn.*, **1993**, *66*, 2965-2971.
6. James, T. D.; Sandanayake, K. R. A. S.; Iguchi, R.; Shinkai, S., *J. Am. Chem. Soc.*, **1995**, *117*, 8982-8987; James, T. D.; Sandanayake, K. R. A. S.; Shinkai, S., *Nature*, **1995**, *374*, 345-346.
7. Suenaga, H.; Arimori, S.; Shinkai, S., *J. Chem. Soc., Perkin Trans. 2*, **1996**,607-612.
8. Fischer, F. C.; Havinge, E., *Recueil*, **1994**, *93*, 21-24; van Riel, H. C. N. A.; Fischer, F. C.; Lugtenburg, J.; Haringa, E., *Tetrahedron Lett.*, **1969**, 3085.
9. Suenaga, H.; Mikami, M.; Sandanayake, K. R. A. S. ; Shinkai, S., *Tetrahedron Lett.*, **1995**, *36*, 4825-4828.
10. Chen, M. -F., *Nucleic acid Res.*, **1983**, *11*, 7231-7250; Idem, *Anal. Biochem.*, **1983**, *130*, 346-352.
11. Kodama, M.; Tagashira, Y.; Imamura, A.; Nagata, C., *J. Biochem.*, **1966**, *59*, 257-264
12. Aoki, I.; Kawabata, H.; Nakashima K.; Shinkai, S., *J. Chem. Soc. Chem. Commun.*, **1991**, 1771-1773; Takeshita M.; Shinkai, S., Chem. Lett., **1994**, 125-128 and references cited therein; Jin, T. ; Ichikawa, K.; Koyama, T., *J. Chem Soc., Chem Commun.*, **1992**, 499-501 and references cited therein.
13. Scatchard, G., *Ann. N. Y. Acad. Sci.*, **1949**, *51*, 660-672.
14. McGhee, D.; Hippel, P. H., *J. Mol. Chem.*, **1974**, *86*, 469-489.
15. Saito, I.; Takayama, M.; Sakurai, T., *J. Am. Chem. Soc.*, **1994**, *116*, 2653-2654.
16. Sugiyama, H.; Kawabata, H.; Fujiwara, T.; Dannoue, Y.; Saito, I., *J. Am. Chem. Soc.*, **1990**, *112*, 5252-5257.
17. Praseuth, D.; Gaudemer, A.; Verlhac, J.-B.; Kraljic, I.; Sissoëff, I.; Guillé, E., *Photochem. Photobiol.*, **1986**, *44*, 717-724.
18. An, Y.-Z.; Chen, C.-H. B.; Anderson, J. L.; Sigman, D. S.; Foote, C. S. ; Rubin, Y., *Tetrahedron* , **1996**, *52*, 5179-5189.

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