Photocleavage of DNA by 4'-Bromoacetophenone Analogs

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4'-Bromoacetophenone analogs, which are able to generate monophenyl radicals capable of hydrogen atom abstraction, were investigated as possible photoinducible DNA cleaving agents. The potential of 4'- bromoacetophenone as a possible new DNA cleaver is explored. Pyrrolecarboxatmid conjugated 4'-bromoacetophenones, in particular, DNA cleaving activity and sequence-selectivity on the contiguous AT base pair sites.

Key words: 4'-Bromoacetophenone, Pyrrolecarboxamide, DNA cleaving agents, Phenyl radical

INTRODUCTION

The design and synthesis of DNA cleaving molecules are currently a topic of intense research investigations. Especially, enediyne anticancer antibiotics in this area has been focused primarily on the design and synthesis of simple enediyne structures which can be mimic their mechanistic feature (Brana *et al.*, 1996; Doyle, *et al.*, 1994; Grissom *et al.*, 1993; Maier, 1999; Nicolaou *et al.*, 1991). Regarding enediyne family as activatable DNA cleaving agents, such complex structures might not be needed but rather that any aryl or vinyl radical would be capable of causing hydrogen atom abstraction reaction from deoxyribose, which initiated the scission of DNA (Griffiths *et al.*, 1992).

However, few efforts have been directed at the investigation of simple phenyl radical species as DNA cleaving agents. Recently, Wender group has been investigated simple photoactivatable DNA cleaving agents represented by benzotriazoles (Wender *et al.*, 1996; Touami *et al.*, 1996) and 4'-bromoacetophenones (Wender, *et al.*, 1999), which could be readily prepared and exhibit potent and selective DNA cleaving activity.

Herein, we report the activity of mono carbon centered radical precursor such as 4'-bromoacetophenone, which can generate a reactive phenyl radical by the photo-induced C-X bond cleavage (Hwang *et al.*, 1992).

In addition, the phenyl radical generating species, 4'-bromoacetophenone, has been attached to pyrrolecarboxamides in order to increase the DNA cleaving ability and DNA sequence selectivity since naturally occurring oligopeptides such as netropsin and distamycin bind to DNA in the minor groove of the helix (Carrondo *et al.*, 1989; Matsumoto *et al.*, 1990; Chen *et al.*, 1992; Edwards *et al.*, 1992). The designed molecules are connected by methylene linker between DNA cleaving moiety and minor groove binders as shown below.

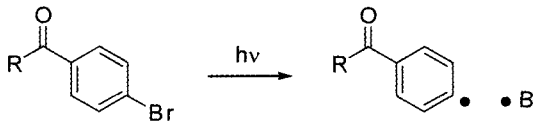

Fig. 1. Photolysis of 4'-bromoactophenone

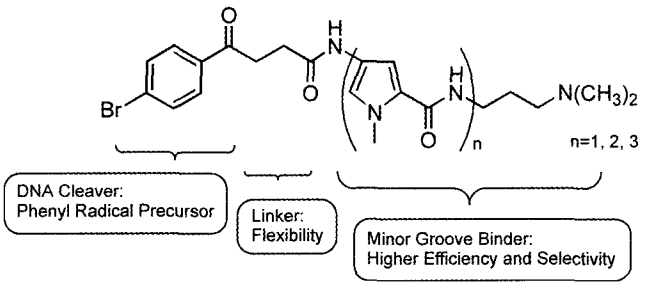

Fig. 2. 4'-Bromoacetophenone-pyrrolecarboxamide conjugates as photoinducible DNA cleaving agents

MATERIALS AND METHODS

Photochemical irradiation was performed with a Hanovia 450W medium pressure mercury arc lamp from Ace

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Glass company. The lamp was suspended in a quartz cooling jacket that was continuously flushed with cold water.

Photolysis of 4'-bromoacetophenones

4'-Bromoacetophenones were dissolved in 20 mL of solvent (concentration=2 mM) in a vycor tube. Before the reaction was started, argon was bubbled through the reaction mixture for 15 min. The tube was then tightly closed with a septum and the reaction was performed under argon atmosphere, room temperature using a Hanovia 450W medium pressure mercury arc lamp, equipped with a Pyrex chamber.

DNA-cleaving experiment

Aqueous solutions of DNA plasmid pBR322 (from SIGMA or USB) or ϕX174RF (from USB) were prepared using a tris buffer at indicated pH. The final concentration of the supercoiled plasmid DNA was equal to 30 μM in base pairs for all experiments reported herein. THF, MeOH, or DMSO solutions of 4'-bromoacetophenone derivatives were added to the DNA solutions and their final concentrations ranged from 40 μM (1.3 eq/bp) to 50 mM. All the solvents were degassed before use. The 20 μL aliquots of the solution of DNA and 4'-bromoacetophenone were placed in microfuge tubes and were irradiated by a Pyrex-filtered light from a Hanovia 450W medium pressure mercury arc lamp for 25 or 30 min. After irradiation was complete, 5 μL of loading buffer was added to reaction and the reactions were loaded onto a 1% agarose gel. The electrophoresis was carried out at 33V for 12-18 h. Gels were stained in a dilute ethidium bromide solution (0.5 μg/mL) for 30 min. The DNA was visualized by means of an UV transilluminator (300 nm UV light source) and photographed with a Polaroid DS 344 camera and Polaroid 667 film.

Preparation of the buffers

Borate running buffer: 108 g of Tris base, 25 g of boric acid and 6.8 g of EDTA were dissolved in deionized water. The volume was brought to 1 l. It was diluted 1: 9 for agarose gel preparation and buffer before use.

DNA buffer (1.0 M, pH 8.0): 5.3 g of Tris base and 8.8 g of TrisHCl were dissolved in deionized water. The volume was brought to 100 mL. It was diluted 1:49 to 20 mM before use.

Preparation of the gels

2.3 g of agarose were dissolved in 230 mL of 10 times-diluted borate running buffer and placed in a 500 mL-erlern. The solution was heated until it got transparent. When the temperature was cooled down to 60°C, the solution was poured into a tray. After 1.5 h, the gel was put in a vat and 10 times-diluted borate running buffer was poured into the vat to cover the gel.

Preparation of 3-^{32}P End Labeled 167 Base Pair Restriction Fragment

Supercoiled pBR322 plasmid (15 μg) was linearized into 4 fragments with 40 U of Eco RI and 60 U of Rsa I restriction endonucleases on 60 μL of React 2 buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, from Gibco BRL). The digest was allowed to proceed at 37°C for 2 h. To this DNA solution was added 5 μL of dTTP, 9 μL of a-32P dATP, and 26 U of Klenow Polymerase. The labeling reaction was incubated at room temperature for 30 min, after which time 8 μL of loading buffer were added, and the mixture directly loaded onto a 2% preparative agarose gel. Electrophoresis was carried out at 180V for 2.5 h. The labeled fragments were visualized by autoradiography; the 167 bp fragment was excised from the gel and recovered by centrifugation through Millipore Ultrafree MC 0.45 μm microcentrifuge filters.

Cleavage and High-Resolution Gel Electrophoresis

2 μL of an aqueous solution of conjugate were added to 18 μL of a solution containing 3-^{32}P labeled restriction fragment and carrier calf thymus DNA (100 μM bp) in 50 mM Tris acetate buffer (pH 7.9). The microfuge tubes containing this reaction mixture were strapped to the outside of a Pyrex photolysis chamber and irradiated for 30 min with light from a 450W medium pressure mercury arc lamp. After photolysis was complete, the DNA was precipitated, and the pellets dissolved in 5 μL of loading buffer. The samples were heat denatured at 95°C for 3 min, chilled immediately on ice, and loaded onto 10% denaturing polyacrylamide gel (7 M urea), in parallel with the Maxam-Gilbert G sequencing reaction. Electrophoresis was carried out at constant power (P=45-55W) for ca. 2 h. Gels were transferred to filter paper and subjected to autoradiography with an intensifying screen at -80°C.

RESULTS AND DISCUSSION

The DNA cleaving activity of 4'-bromoacetophenone was determined by monitoring their effectiveness in converting circular supercoiled DNA (form I) to circular relaxed DNA (form II) and linear DNA (form III). 4'-bromoactophenones were irradiated at various concentrations for 30 min in the presence of ϕX174RFI DNA (30μM/bp) in 1:9 DMSO:Tris buffer (20 mM, pH 7.5).

The concentrations of the 4'-bromoacetophenone were 0.1, 1, 10,and 100mM. 4'-Bromoacetophenone formed form III at 10 mM with complete disappearance of form I.

The inhibitory effect of radical scavengers was also examined to confirm the attribution of carbon centered radical to DNA cleaving activity. TEMPO, known as a carbon centered radical scavenger, and sodium benzoate, a hydroxyl radical scavenger, were added into the reaction mixtures for the DNA cleavage assay. The DNA

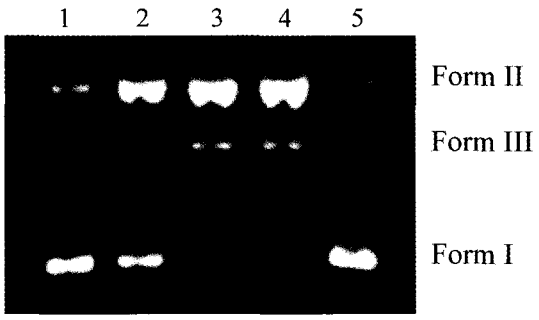

Fig. 3. Light induced cleavage of DNA by 4'-bromoacetophenone. Supercoiled DNA (ϕX174RF) runs at position I, nicked DNA at position II, and linear DNA at position III. Unless otherwise indicated, all DNA cleavage reactions were irradiated with Pyrex-filtered light from a 450W medium pressure mercury arc lamp for 30 min at 25°C. Lane 1-4, DNA (30 μM/bp) +4'-bromoacetophenone at concentrations of 0.1, 1, 10, and 100 mM, respectively; lane 5, control ϕX174RF DNA +4'-bromoacetophenone (100 mM), no hv.

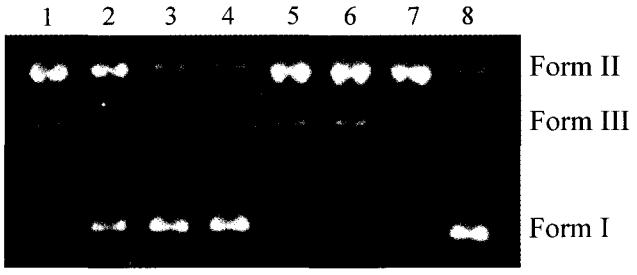

Fig. 4. The effect of radical scavenger on light induced cleavage of DNA by 4'-bromoacetophenone. Supercoiled DNA (ϕX174 RF) runs at position I, nicked DNA at position II, and linear DNA at position III. Unless otherwise indicated, all DNA cleavage reactions were irradiated with Pyrex-filtered light from a 450W medium pressure mercury arc lamp for 30 min at 25 °C. Concentration of the 4'-bromoacetophenone was 10 mM for each reaction. Lane 1, control ϕX174RF DNA (30 μM/bp) +4-bromoacetophenone; lanes 2-4, DNA +4-bromoacetophenone +TEMPO at concentrations of 10 mM, 50 mM, and 200 mM, respectively; lanes 5-7, DNA +4-bromoacetophenone + Sod. benzoate at concentrations of 10 mM, 50 mM, and 200 mM, respectively. ;Lane 8, control DNA+haloacetophenone, no hv.

cleaving activity of 4'-bromoacetophenone was decreased as the concentration of TEMPO was increased while its activity was not affected by sodium benzoate (Fig. 4). This result indicates that phenyl radical acts directly to cleave DNA.

DNA cleaving activity of pyrrolecarboxamide conjugated 4'-bromoacetophenones, **1-3**, was tested. As we expected from the previous study (Touami *et al.*, 1997), the DNA cleaving activity was remarkably enhanced by DNA recognition moiety, pyrrolecarboxamides, depending on the number of pyrrole unit as well as the concentrations of the compounds (Fig. 5).

The concentration of the 4'-bromoacetophenone-

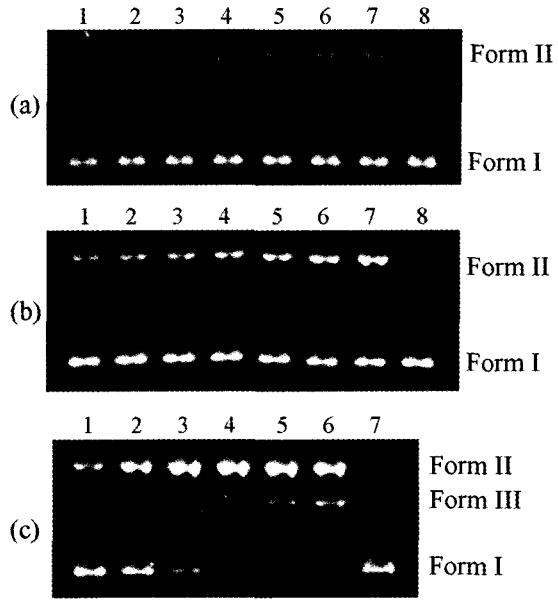

Fig. 5. Light induced cleavage of DNA by peptide linked 4'-bromoacetophenones **1**, **2** and **3**. Supercoiled DNA (ϕX174RF) runs at position I, nicked DNA at position II, and linear DNA at position III. Unless otherwise indicated, all DNA cleavage reactions were irradiated with Pyrex-filtered light from a 450 W medium pressure mercury arc lamp for 30 min at 25°C. (a) Lane 1-7, DNA (30 μM/bp)+**1** at concentrations of 3 μM, 10 μM, 20 μM, 30 μM, 50 μM, 100 μM, and 200μM, respectively; lane 8, control ϕX174RF DNA+**1** (200 μM), no hv. (b) Lane 1-7, DNA (30 μM/bp)+**2** at concentrations of 3 μM, 10 μM, 20 μM, 30 μM, 50 μM, 100 μM, and 200μM, respectively; lane 8, control ϕX174RF DNA+**2** (200 μM), no hv. (c) Lane 1-6, DNA (30 μM/bp)+**3** at concentrations of 3 μM, 10 μM, 20 μM, 30 μM, 50 μM, and 100 μM, respectively; lane 7, control ϕX174RF DNA+**3** (200 μM), no hv.

linked pyrrolecarboxamide was ranged to 3 to 200 μM to compare their DNA cleaving activity. Form III DNA was observed at 30 μM concentration of distamycin type analog **3**. The 3 μM reaction of distamycin analog **3** gave higher activity even than those of the 20 μM netropsin analog **2** with complete disappearance of form I at concentrations above 20 μM. The DNA cleaving activity of acetophenone analog which has no bromine substituent on the phenyl ring was also tested (data not shown). At high concentration (1 mM) of acetophenone analog, the cleaving activity was barely recognizable, emphasizing the role of the phenyl bromide as a phenyl radical precursor.

The cleavage selectivity of the pyrrolecarboxamide linked 4'-bormoacetophenone derivatives was determined by sequencing analyses of the DNA cleavage products obtained when compounds **1-3** were photolyzed in the presence of a 3-^{32}P labeled 517 base pair restriction fragment from pBR322 (Pichersky, 1993). As expected for a cleaving agent bound to a distamycin or netropsin analog, the cleavage intensities are the highest

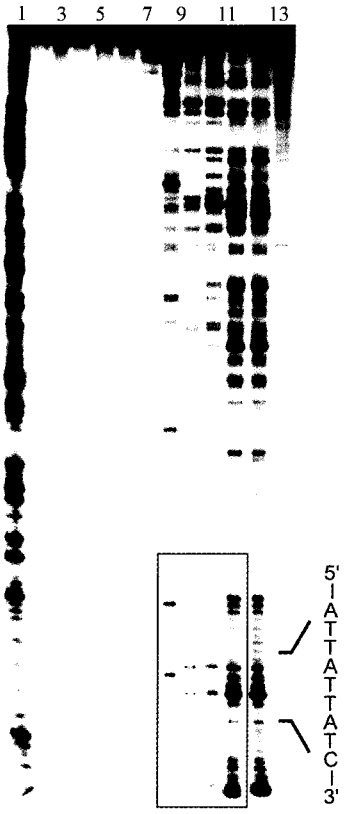

Fig. 6. Autoradiogram of 8% denaturing gel polyacrylamide gel showing cleavage of 3'-^{32}P end-labeled 517 base pair restriction fragment (EcoRI/RsaI) from pBR322 by peptide linked bromoacetophenone **1**, **2**, and **3**. All reactions were irradiated with Pyrex-filtered light from a 450W medium pressure mercury arc lamp for 30 min at 25°C. The cleaving site is shown to the right of the autoradiogram. Lane 1, Maxam-Gilbert G reaction; Lane 2, DNA control; lanes 3-5, DNA+**1** at concentrations of 10 μM, 50 μM, and 200 μM, respectively; lanes 6-8, DNA+**2** at concentrations of 10 μM, 50 μM, and 200 μM, respectively; lanes 9-12, DNA+**3** at concentrations of 5 μM, 15 μM, 50 μM, and 100 μM, respectively; lane 13, DNA+4-bromoacetophenone (500 μM).

in AT-rich regions of the DNA. A significant cleaving site is marked to the right of the autoradiogram, which was quantified by densitometry, and this data was used to construct histograms for the DNA cleavage observed in the lower regions of the autoradiograms (data not shown). The histograms show that compounds **2** and **3** produce cleavage within and adjacent to sites of multiple contiguous AT base pairs and the cleavage pattern remains at high concentration. The cleavage assay and sequencing of these compounds showed remarkable correlation between chain lengths and activities leading to the highest affinity of distamycin type analog **3** to bind to DNA and cleave it.

In summary, the potential of 4'- bromoacetophenone and the 4'-bromoacetophenone-pyrrolecarboxamide conjugates as novel sequence specific DNA cleavers has been investigated. These novel DNA cleavers showed potent DNA cleaving activities. Especially, the 4'-bromoacetophenone-pyrrolecarboxamide conjugates showed remarkable DNA cleaving activity at even micromolar concentrations of the reagents and sequence-selectivity on the contiguous AT base pair sites. The DNA binding affinity increases as expected as the number of polyamide increases. Therefore, the use of 4'-bromoacetophenone species as phenyl radical precursors thus offers a lead into the design of new DNA cleaving agents. Furthermore, conjugation of the simple radical precursor, 4-bromoacetophenone, with various polyamides will facilitate the design of new DNA photocleaver targeted for other sequences.

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