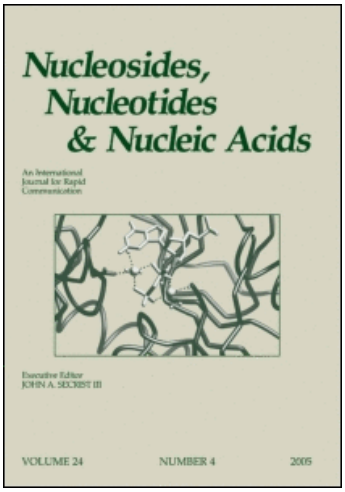

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 09 August 2003

To cite this Article Koval, O. A. , Oleinikova, S. B. , Chernolovskaya, E. L. , Litvak, V. V. and Vlassov, V. V.(2003) 'New Reagent for Protein-DNA Contacts Footprinting', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 1587 — 1589
To link to this Article: DOI: 10.1081/NCN-120023040
URL: http://dx.doi.org/10.1081/NCN-120023040

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1587–1589, 2003

New Reagent for Protein-DNA Contacts Footprinting

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ABSTRACT

We have found, that the reaction of *o*-bromobenzoic acid with Cu^{2+} ions can be used as a source of activated oxygen species capable of cleaving DNA. Possibility to apply this reaction for footprinting the nucleosome core in the reconstituted chromatin was demonstrated.

Key Words: Protein-DNA footprinting; Active oxygen species; DNA cleavage; Cu^{2+} ions; *o*-Bromobenzoic acid.

Small reagents capable of cleaving DNA under physiological conditions find applications in design of therapeutics and are used as probes in footprinting experiments. Metallocomplexes, generating activated oxygen species, EDTA-Fe^{2+}, phenanthroline-Cu^{2+}, ascorbic acid/Cu^{2+} and few related copper system, are used for investigation of the structure of nucleic acid and nucleic acid-protein complexes.[1] Recently, we have found that the *o*-bromobenzoic acid/Cu^{2+} system in the presence of O_2 can efficiently cleave DNA under physiological conditions and does not require H_2O_2 or reducing agents.[2] Alkoxyl radicals were identified as the active species in the reaction, using ESR spine trapping techniques. In this report we demonstrate,

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DOI: 10.1081/NCN-120023040
1525-7770 (Print); 1532-2335 (Online)

that *o*-bromobenzoic acid with Cu^{2+} ions can be used for footprinting DNA-protein complexes.

Reaction with DNA

At 37°C substantial cleavage of DNA by *o*-bromobenzoic acid (**obb**) with Cu^{2+} ions occurs within 6 h. Some DNA scission occurs even without piperidine treatment. Piperidine treatment results in more pronounced DNA degradation suggesting damage or removal heterocyclic bases by the *o*-bromobenzoic acid/Cu^{2+} system. Single stranded oligonucleotides are cleaved more efficiently then the double stranded oligonucleotides. The cleavage patterns demonstrate no sequence selectivity.

Footprinting of a DNA-Protein Complex

We have tested the **obb**/Cu^{2+} system as a footprinting probe using reconstituted nucleosomes as a model. The DNA was a [^{32}P]-labeled 352 bp *c-fos* promoter region

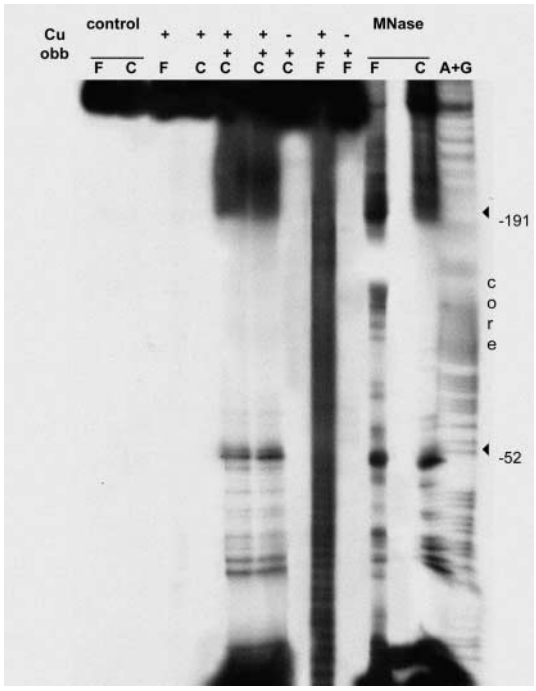

Figure 1. Cleavage of the free (**F**) and reconstituted (**C**) 352 bp DNA fragment by the *o*-bromobenzoic acid/Cu^{2+} system. Control – **F** and **C** incubated in the reaction buffer (50 mM imidazole, pH 7.0). MNase – **F** and **C** treated with 0.02 unit micrococcal endonuclease for 5 min; The samples were incubated with 10 μM **obb** and 10 μM $\mathbf{Cu^{2+}}$ for 24 h at 37°C. A + G – specific Maxam-Gilbert reaction. Arrows indicate the position of nucleosomal core.

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DNA fragment positions from −348 to +3. Reconstitution of nucleosomes was performed by high salt exchange method, using natural mononucleosomes from human placenta chromatin. It is known that nucleosomal chain consist of "core" particle of 146 bp of DNA that is tightly associated with the histone octamer, and 30–35 bp of "linker DNA". The reconstituted fragment and free DNA fragment were incubated with *o*-bromobensoic acid and Cu^{2+}. The cleavage patterns of the free DNA (**F**) and the reconstituted fragment (**C**) shown in Fig. 1. It is seen, that in the nucleosome core DNA is protected from the scission, while the linker DNA is cleaved. Similar protection patterns were observed in the experiment, when micrococcal endonuclease was used as a probe.

Results of this study demonstrate, that *o*-bromobenzoic acid with Cu^{2+} ions can be used as a reagents for footprinting of protein-DNA complexes.

ACKNOWLEDGMENTS

The research was supported by award No. REC-008 from CRDF (U.S.A.) and by Russian Government Program "Gene-directed biologically active compounds".

REFERENCES

1. Sigman, D.; Mazumder, A.; Perrin, D. Chemical nucleases. Chemical Reviews **1993**, *93*, 2295–2316.
2. Koval, O.; Chernolovskaya, E.; Litvak, V.; Vlassov, V. Cu^{2+}-dependent DNA-cleaving activity of arenas. Nucleosides, Nucleotides & Nucleic Acids **2001**, *20*, 851–854.

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