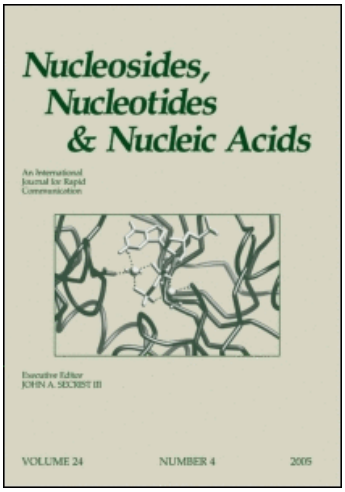

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Cu^2-DEPENDENT DNA-CLEAVING ACTIVITY OF ARENES

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Cu^{2+}-DEPENDENT DNA-CLEAVING ACTIVITY OF ARENES

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ABSTRACT

In the presence of Cu(II) ions, plasmid DNA is cleaved under physiological condition by different arenes at low concentrations. The cleavage was dependent on the presence of O_2. The DNA cleavage efficiency of the designed system arene-Cu is comparable to that of the well-known DNA cleaving reagents such as phenanthroline-Cu and ascorbic acid-Cu. However in contrast to the mentioned reagents, the system arene-Cu does not require external reducing agents or H_2O_2.

A number of metal complexes capable of inducing DNA damage have been developed. Bleomycin-iron, phenanthroline-Cu, ascorbic acid - Cu and few related copper complexes act like catalysts causing oxidative damage to nucleic acids [Sigman, 1993]. Some of metal complexes were used as reactive groups in oligonucleotide conjugates designed for cleaving nucleic acids. These conjugates were shown to cleave complementary DNA, although with low efficacy and in conditions far from physiological conditions, usually in the presence of a reducing agent or H_2O_2. Sequence-directed cleavage of DNA has been achieved by means of complementary oligonucleotides bearing terminal reactive group. Some metal complex systems, for instance, ascorbic acid-Cu are not sutable for conjugation with oligonucleotides. The goal of our study is the development of chemical cleavage systems that are easily amenable to chemical synthetic manipulation

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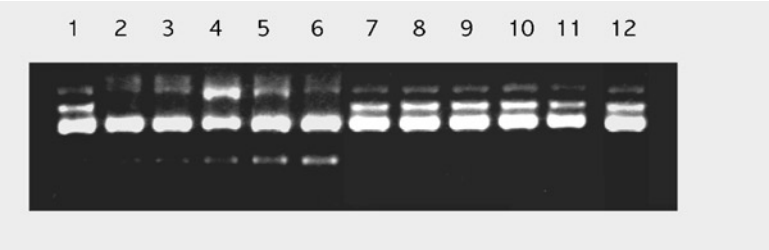

Figure 1. Agarose gel analysis of plasmid DNA cleaved by compounds **1–5**. Plasmid DNA was incubated at 37°C for 1h with or without 10 μM compounds **1–5** and 10 μM $CuSO_4$. Lane 1-DNA without reagents; lane 2–6 DNA incubated with compounds **1–5** respectively; lane 7–11 DNA incubated with compounds **1–5** respectively without $CuSO_4$; lane 12-plasmid DNA incubated with $CuSO_4$.

and have suitable biocompatability. We propose to use as DNA-cleaving groups complexes of arenes with Cu. These complexes are unique in that arenes are small simple compounds and can be easily modified to change their reactivity. A variety of simple and rather inert arenes that react with nucleophiles in the presence of specific metal ions can be easily conjugated to oligonucleotides and peptides.

We have investigated cleavage of plasmid DNA by a simple arene-Cu system under physiological conditions. In order to assess the competence of *o*-bromobenzoic acid (**1**), *o*-chlorobenzoic acid (**2**), *p*-bromobenzoic acid (**3**), *p*-chlorobenzoic acid (**4**) and salicylic acid **(5)** for DNA strand scission, each of the compounds was incubated with DNA under identical reaction conditions. We have found that the compounds cleave DNA in the presence of Cu(II) at concentration as low as 10 μM (lane 2, Fig. 1). In the absence of Cu(II) ions , arenes are inert to the DNA (lane 7–11, Fig. 1) up to 10 mM concentration. Figure 1 show that all the compounds display similar DNA cleaving reactivity.

To identify the groups in the structure of the tested arenes that are essential for the cleavage reaction, we tested the effect on DNA of compounds **1–5** and phenol, benzoic acid, bromobenzene and sodium acetate. Only benzoic acid cleaved DNA as well as **1–5** (date not shown). These results indicate that both an aromatic ring and a carboxyl group are necessary for effective cleavage reaction, which suggests that both π and σ coordination may be necessary. We have found that all the investigated compounds can cleave plasmid DNA to some extent without addition of Cu(II) if their concentrations are higher then 0.01 M. An explanation could be that in this case the natural plasmid bound copper ions participated in the reactions, because addition of Na_2EDTA strongly inhibited cleavage of DNA. This fact indicates that both Cu(II) and arenes are essential for the DNA damaging reaction. No cleavage occurred when the plasmid DNA was incubated with Cu(II) at millimolar concentration in the absence of the compounds under investigation.

To elucidate the mechanism of the reaction we have investigated the potential role of oxygen in the process of DNA cleavage. The reaction was performed under

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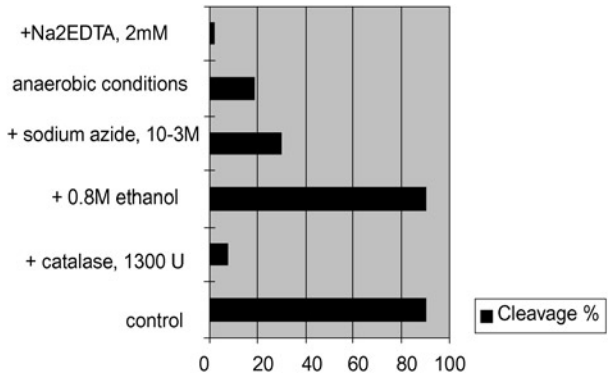

Figure 2. Plasmid DNA cleavage by *o*-bromobenzoic acid under anaerobic conditions and with oxygen species scavangers. Cleavage reactions were carried out for 3 h at 37°C. Reaction mixtures contained 10 μM o-bromobenzoic acid, 10 μM $CuSO_4$ and other components as indicated in the figure. To provide anaerobic condition all mixture components were degassed for 20 min by argon before the reaction.

normal atmospheric conditions, under anaerobic conditions and in the presence of various reactive oxygen species scavengers. The data presented in Figure 2 demonstrate that cleavage by Cu(II)-arylcarboxylate does not occur under anaerobic condition. The obvious role of O_2 might be in the generation of oxygen radicals such as .OH, which could be the actual mediators of DNA destruction [Pogozelsski, 1998]. As shown in Figure 2, singlet oxygen scavenger, sodium azide is also much more effective as an inhibitor than the OH radical's scavenger, alcohol. Additional evidence for oxygen dependence of the cleavage in the presence of arenes and Cu(II) ions was obtained in the experiment with catalase, an enzyme that disproportionate H_2O_2 to yield $H_2O + O_2$. The enzyme completely inhibits the cleavage (Fig. 2). Therefore it can be concluded, that both H_2O_2 and singlet oxygen participate in the reaction.

Generation of active oxygen species can occur as a result of changes of oxidizing state Cu^{II}/Cu^{I} or Cu^{II} / Cu^{III} [Yamomoto, 1989;]. To find out, what active species participate in the DNA damage, the effect of reducing or oxidizing agents H_2O_2 and β-mercaptoethanol on the DNA cleavage induced by Cu(II)-arenes was investigated. As shown in Figure 3, addition of H_2O_2 stimulates the cleavage, while addition of β-mercaptoethanol does not effect the process.

This result indicates that the DNA cleavage reaction by arenes with Cu^{II} is more likely accompanied by transition of the copper oxidizing effect Cu^{II}/Cu^{III} then Cu^{II}/Cu^{I}. We have compared the cleavage efficacy of the system *o*-bromobenzoic acid-Cu with those of the well-known DNA cleaving reagents: phenanthroline-Cu and ascorbic acid-Cu. Figure 3 demonstrates that the efficiency of DNA cleavage without additional agent in the system *o*-bromobenzoic acid-Cu is comparable with that for ascorbic acid-Cu. Under identical condition cleavage does not occure in the system phenanthroline-Cu without addition of β-mercaptoethanol.

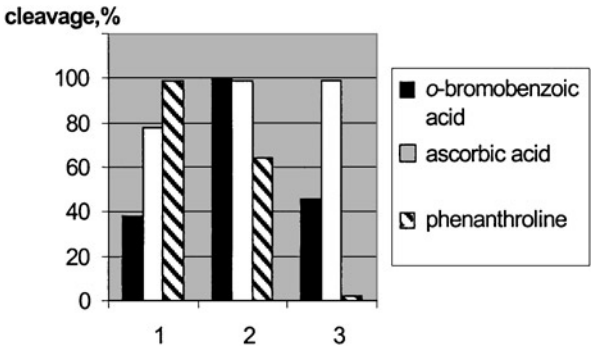

Figure 3. Cleavage of plasmid DNA by metal complexes. Effect of β-mercaptoethanol and H_2O_2. Cleavage reaction were carried out for 2 h at 37°C. Reaction mixtures contained 10 μM $CuSO_4$, 10 μM reagents, and 1 mM 2-mercaptoetanol or 0.01mM H_2O_2. 1 - cleavage with 2 - mercaptoetanol, 2-cleavage with H_2O_2, 3 - cleavage without additional factor.

We have demonstrated that arenes of different structure containing carboxyl groups can effectively cause cleavage of DNA in the presence of Cu under physiological condition. These reagents can be used for the design of a new class of oligonucleotide conjugates, affinity reagents for inactivating viral nucleic acids and specific oncogenes.

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