Function of Cupric Ion in the Breakage of pBR322 ccc-DNA by D-Glucosamine

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The function of Cu^{2+} in the breakage of pBR322 ccc-DNA by D-glucosamine was investigated using agarose gel electrophoresis. The maximal stimulatory effects on the DNA breakage and $\cdot\text{OH}$ production were observed at the same concentration of Cu^{2+} in an aqueous solution of D-glucosamine. An aqueous solution of D-glucosamine containing 1 mM of Cu^{2+} generated less O_2^- and H_2O_2 than $\cdot\text{OH}$. The generation of these active oxygens, either in the presence or absence of Cu^{2+} , was affected little by the addition of DNA. Superoxide dismutase inhibited the DNA-breaking action of D-glucosamine in the absence, but not the presence, of Cu^{2+} . Hydroxyl radical-scavengers inhibited the reaction in the presence as well as the absence of Cu^{2+} . The reaction in the presence of Cu^{2+} was inhibited by d-GMP and a different kind of DNA. An atomic absorption analysis showed that copper binding to pBR322 ccc-DNA was concentration-dependent. Cupric ions were found to bind to the ccc-DNA, and were found to stimulate the autoxidation of D-glucosamine *via* generation of active oxygen molecules, including, especially $\cdot\text{OH}$.

In our previous studies, we have found that some aminosugars including D-glucosamine cause the breakage of PL-1 phage¹⁾ and pBR322 plasmid²⁾ DNA, and that this reaction is stimulated by Cu^{2+} . In this study, therefore, we have investigated in detail the function of Cu^{2+} in the breakage of DNA by aminosugars using a system including plasmid pBR322 and D-glucosamine.

Materials and Methods

Plasmid DNA and other chemicals. Covalently closed, circular duplex DNA (ccc-DNA) of pBR322 and pUC9 were isolated from *Escherichia coli* W3350/pBR322 and *Escherichia coli* C600S/pUC9, respectively, as described, in principle, by Kupersztoch-Portony *et al.*³⁾ Linear DNA of pFP6 was prepared as follows. pBR322 DNA and pUC9 DNA, both digested with *Eco*RI and *Pst*I, were ligated using T4 DNA ligase. Competent cells of *Escherichia coli* C600S were transformed with the ligated DNA fragments, and ampicillin resistant-transformants were selected. Then, a transformant containing the 3399-bp plasmid DNA (752 bp fragment of pBR322 plus 2647 bp fragment of pUC9) was selected. The 3399-bp plasmid

DNA was digested with *Bam*HI to produce the linear DNA. This DNA, named pFP6 linear DNA, could be clearly distinguished from ccc-, open circular (oc-), and linear DNA of pBR322 by agarose gel electrophoresis.

Nitroblue tetrazolium hydrochloride, 3-[methylthio]-propionaldehyde, bovine blood type I superoxide dismutase (3,100 units/mg protein), and bovine liver catalase (3,765 units/mg protein) were purchased from Sigma Chemical. Ethylene (99.5%) was purchased from Gasu-kuro-Kogyo Co. The other chemicals and enzymes were purchased from Wako Jun-yaku and Nakarai-Kagaku Co.

Reaction of pBR322 ccc-DNA with D-glucosamine. The reaction mixture (100 μl) containing 1 μg of pBR322 ccc-DNA, 0.1 M D-glucosamine, and 50 mM Tris-HCl buffer (pH 7.2) was incubated at 37°C. At intervals, 10 μl of this solution was mixed with 3 μl of a 22.5 mM EDTA solution (pH 8.2) containing 1.5% (w/v) sodium dodecyl sulfate, 25% (w/v) sucrose and 0.02% (w/v) bromophenol blue, and the resultant mixture was electrophoresed on an agarose gel. The electrophoretic procedure and measurement of each DNA (ccc-, oc-, linear-) on the gels were described in our previous paper.²⁾

Measurement of active oxygen molecules. The superoxide (O_2^-) concentration was measured by the method of

Beauchamp and Fridovich.⁴⁾ A mixture of D-glucosamine and Cu^{2+} was incubated with $10\ \mu\text{M}$ nitroblue tetrazolium hydrochloride at 37°C in 50 mM Tris-HCl buffer (pH 7.2) containing 0.1 mM EDTA, and once per minute the OD at 560 nm of the mixture was measured to obtain the $\Delta\text{OD}_{560\text{ nm}}/\text{min} \times 100$.

The hydrogen peroxide (H_2O_2) concentration was measured by the method of Fujimoto.⁵⁾ A mixture of D-glucosamine and Cu^{2+} was incubated at 37°C in 50 mM Tris-HCl buffer (pH 7.2) with shaking. At intervals, 0.7 ml of this solution was mixed with 2.8 ml of 1% titanium sulfate in 2.5 N H_2SO_4 and the OD at 410 nm was measured. The amounts of H_2O_2 (mM) were then calculated from a standard calibration curve for H_2O_2 .

The hydroxyl radical ($\cdot\text{OH}$) concentration was measured by the method of Beauchamp and Fridovich.⁶⁾ A mixture of D-glucosamine and Cu^{2+} was incubated with 1 mM 3-[methylthio]propionaldehyde at 37°C in 50 mM Tris-HCl buffer (pH 7.2) in an Erlenmeyer flask sealed with a serum cap and shaken. At intervals, 1 ml of the gas phase above the mixture was analyzed for ethylene on a Yanako G1800 gas chromatograph equipped with a glass column of Chromosorb 102 and a flame ionization detector using N_2 as the carrier gas. The amounts of ethylene (ppm) were calculated from the peak area at the retention time of 43 sec on the chromatogram using a standard calibration curve for ethylene.

Measurement of Cu. Cu was measured using a Hitachi 180-50 atomic absorption spectrophotometer.

Results

Effects of Cu^{2+} on the strand breakage of ccc-DNA by D-glucosamine

Figure 1 shows the course of the reaction of pBR322 ccc-DNA with 0.1 M D-glucosamine in the presence or absence of 1 mM Cu^{2+} . In the control condition, without Cu^{2+} , the amount of ccc-DNA decreased linearly and slowly with time, and the equivalent amount of oc-DNA appeared. No linear DNA appeared, even after 6 hr of incubation. However, in the presence of Cu^{2+} , all the ccc-DNA was converted into linear DNA *via* oc-DNA, and, after a prolonged reaction, the resultant linear DNA was further fragmented. Cupric chloride alone at this concentration did not affect the reaction. These results indicate that the D-glucosamine was primarily responsible for the single-strand breakage in the ccc-DNA, and

Fig. 1. Course of the Strand Breakage of pBR322 ccc-DNA by D-Glucosamine.

pBR322 ccc-DNA was incubated with 0.1 M D-glucosamine at 37°C in 50 mM Tris-HCl buffer (pH 7.2) with (—) or without (---) 1 mM CuCl_2 . At intervals, the amounts of ccc-DNA, oc-DNA, and linear DNA separated from each other by agarose gel electrophoresis were measured.

Symbols: ●, ccc-DNA; ●, oc-DNA; ○, linear DNA.

that this reaction was stimulated by Cu^{2+} . The DNA strand breakage-stimulating activity of Cu^{2+} was maximum at the concentration of 1 mM, and, at higher concentrations, its activity decreased.

Effects of Cu^{2+} on the generation of active oxygens in aqueous solutions of D-glucosamine

The effects of Cu^{2+} on the generation of active oxygens such as O_2^- , H_2O_2 , and $\cdot\text{OH}$ in the aqueous solution of D-glucosamine with or without DNA was then studied, since Cu^{2+} is known to catalyze the autoxidation of some reducing compounds to generate these active oxygens in aqueous, oxygen-containing solutions.^{7,8)} As Table I shows, these three kinds of active oxygens were generated in the aque-

Table I. EFFECTS OF Cu^{2+} ON THE GENERATION OF O_2^- , H_2O_2 , AND $\cdot\text{OH}$ IN AN AQUEOUS SOLUTION OF D-GLUCOSAMINE

O_2^- , H_2O_2 , and $\cdot\text{OH}$ generated in the Cu^{2+} -containing aqueous solution of 0.2 M D-glucosamine with or without DNA were measured by the methods described in Materials and Methods. In parentheses are shown their relative amounts to that without Cu^{2+} and DNA.

Active oxygens	Concn. of Cu^{2+} (mM)	Amounts generated		
		DNA ($\mu\text{g}/\text{ml}$)		
		0	10	100
O_2^- ($\Delta\text{OD}_{560\text{nm}}/\text{min} \times 100$)	0	0.37 (1.0)	0.29 (0.8)	0.28 (0.8)
	0.001	0.51 (1.4)	0.33 (0.9)	0.43 (1.2)
	0.01	0.80 (2.2)	0.35 (1.0)	0.44 (1.2)
	0.1	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)
	1	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)
	10	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)
H_2O_2 (mM)	0	0.97 (1.0)	0.84 (0.9)	0.65 (0.7)
	0.001	1.06 (1.1)	1.00 (1.0)	0.87 (0.9)
	0.01	0.81 (0.8)	0.69 (0.7)	0.37 (0.4)
	0.1	0.21 (0.2)	0.17 (0.2)	0.02 (0.0)
	1	0.08 (0.0)	0.07 (0.0)	0.00 (0.0)
	10	0.08 (0.0)	0.08 (0.0)	0.00 (0.0)
$\cdot\text{OH}$ (ppm)	0	13 (1.0)	11 (0.9)	14 (1.1)
	0.001	32 (2.5)	36 (2.8)	53 (4.1)
	0.01	146 (11.2)	148 (11.4)	176 (13.5)
	0.1	261 (20.0)	250 (19.2)	280 (21.5)
	1	334 (25.7)	307 (23.6)	344 (26.5)
	10	285 (21.9)	305 (23.5)	310 (23.9)

ous solution of D-glucosamine even in the absence of Cu^{2+} . However, the amount of $\cdot\text{OH}$ increased greatly as the concentration of Cu^{2+} increased, up to 1 mM. At higher concentrations of Cu^{2+} , the amounts of $\cdot\text{OH}$ generated decreased. On the other hand, the concentrations of Cu^{2+} that yielded the maximal amounts of O_2^- and H_2O_2 were 0.01 and 0.001 mM, respectively. The amounts of these active oxygens generated, however, were only a little higher than those generated in the absence of Cu^{2+} . At 1 mM Cu^{2+} , little of either O_2^- or H_2O_2 could be detected. The concentration of Cu^{2+} which yielded the maximal amounts of $\cdot\text{OH}$ matched the Cu^{2+} concentration needed to stimulate the D-glucosamine-induced strand-breakage of DNA (see Table II). The generation of these active oxygens, either in the presence or absence of Cu^{2+} , was not affected much by the addition of DNA. The apparent, slight decrease in the case of H_2O_2 was found to be due to a preventative effect of DNA on the reaction between H_2O_2 and TiSO_4 (data not presented in this paper).

Effects of enzymes and radical scavengers on the D-glucosamine-induced DNA strand-breakage in the presence of Cu^{2+}

Table III shows the effects of some en-

Table II. EFFECTS OF Cu^{2+} ON THE STRAND BREAKAGE OF pBR322 ccc-DNA BY D-GLUCOSAMINE

Plasmid pBR322 ccc-DNA was incubated with 0.2 M D-glucosamine at 37°C for 3 hr in 50 mM Tris-HCl buffer (pH 7.2) in the presence of the indicated concentrations of CuCl_2 .

CuCl_2 (mM)	Relative amounts of DNA (%)		
	ccc-	oc-	linear
Control ^a	78.6	21.4	0
0	36.8	63.2	0
0.1	34.3	65.7	0
1.0	0	71.3	28.7
10.0	0	90.8	9.2

^a pBR322 ccc-DNA was incubated neither with CuCl_2 nor D-glucosamine.

Table III. EFFECTS OF ENZYMES ON THE STRAND BREAKAGE OF pBR322 ccc-DNA BY D-GLUCOSAMINE

pBR322 ccc-DNA (1 μ g) was incubated with 0.1 M D-glucosamine at 37°C in 50 mM Tris-HCl buffer (pH 7.2) in the presence of enzymes for 3 hr with or without 1 mM CuCl₂. Percent inhibition of the DNA strand breakage was computed from the amounts of ccc-DNA and linear DNA as follows: Inhibition (%) = $\{[(C_1 - L_1) - (C - L)] / [C_0 - (C - L)]\} \times 100$, where C_1 and L_1 are the amounts of remaining ccc-DNA and linear-DNA of the system with inhibitor, C and L those of a control system without inhibitor, and C_0 is that of remaining ccc-DNA of another control system with neither inhibitor nor D-glucosamine. In parentheses are shown the data with their respective enzymes which were boiled previously for 10 min.

Enzymes	Concn. (μ g/ml)	Inhibition (%)	
		Cu ²⁺ (-)	Cu ²⁺ (+)
SOD ^a	0.1	14 (-3)	-1 (2)
	1.0	41 (10)	0 (1)
	10	49 (10)	0 (0)
	100	52 (33)	0 (1)
Catalase	0.1	13 (-39)	2 (-1)
	1.0	18 (-4)	1 (-1)
	10	91 (-31)	31 (2)
	100	98 (-39)	80 (1)
SOD ^a +Catalase each	0.1	15 (2)	2 (2)
	1.0	56 (9)	1 (1)
	10	94 (26)	34 (0)
	100	94 (24)	82 (3)

^a Superoxide dismutase.

zymes on the D-glucosamine-induced DNA strand-breakage either in the presence or absence of 1 mM Cu²⁺. Superoxide dismutase, which causes disproportionation in O₂⁻ to yield H₂O₂, weakly inhibited the reaction in the absence of Cu²⁺. However, inhibition of the reaction by the heat-inactivated superoxide dismutase demonstrated a nonspecific inhibitory action of the enzyme protein on the inhibitory effect of superoxide dismutase. On the other hand, in the presence of Cu²⁺, superoxide dismutase did not affect the strand-breaking reaction. Catalase inhibited the reaction even in the presence of Cu²⁺, although its inhibitory effect was somewhat weaker than that in the absence of Cu²⁺. The activity of catalase and superoxide dismutase, together,

Table IV. EFFECTS OF RADICAL SCAVENGERS ON THE STRAND BREAKAGE OF pBR322 ccc-DNA BY D-GLUCOSAMINE

pBR322 ccc-DNA (1 μ g) was incubated with 0.1 M D-glucosamine at 37°C in 50 mM Tris-HCl buffer (pH 7.2) in the presence of radical scavengers for 3 hr with or without 1 mM CuCl₂. Percent inhibition of the DNA strand breakage was computed as shown in the footnote of Table III. Corresponding active oxygens are shown in parentheses.

Additions	Concn. (mM)	Inhibition (%)	
		Cu ²⁺ (-)	Cu ²⁺ (+)
Tiron ^a (O ₂ ⁻)	0.1	4	0
	1.0	70	0
	10	104	1
	100	—	96
KI (·OH)	1.0	8	0
	10	22	16
	100	58	48
Thiourea (·OH)	1.0	0	15
	10	10	39
	100	83	94
HCOONa (·OH)	1.0	0	8
	10	8	9
	100	29	28
D-Mannitol (·OH)	1.0	9	10
	10	13	12
	100	30	17
DMSO ^b (·OH)	1.0	1	0
	10	13	6
	100	66	16
DABCO ^c (¹ O ₂)	1.0	-27	4
	10	-46	-7
	100	-59	-27

^a 4,5-Dihydroxy-1,3-benzendisulfonic acid.

^b Dimethyl sulfoxide.

^c 1,4-Diazabicyclo[2.2.2]octane.

was not more than the activity of catalase alone. As Table IV shows, among the radical scavengers, Tiron, which scavenges O₂⁻, inhibited the reaction most effectively; its inhibition of the reaction was more effective in the absence than in the presence of Cu²⁺. This may have been due to a possible chelating action of Tiron on metal ions.⁹⁾ Some hydroxyl radical-scavengers, such as KI, thiourea, HCOONa, D-mannitol, and dimethyl sulfoxide, inhibited the reaction in the pres-

Fig. 2. Effects of pFP6 DNA on the Strand Breakage of Plasmid pBR322 ccc-DNA by D-Glucosamine. pBR322 ccc-DNA (0.1 μg) was incubated with 0.1 M D-glucosamine at 37°C in 50 mM Tris-HCl buffer (pH 7.2) in the presence of various concentrations of pFP6 DNA for 3 hr with (—) or without (---) 1 μM CuCl_2 . Symbols: ●, ccc-DNA; ◐, oc-DNA; ○, linear DNA.

ence as well as the absence of Cu^{2+} . DABCO, an $^1\text{O}_2$ quencher, accelerated the reaction, although the reason for this effect remains unclear. These results suggest that Cu^{2+} may change O_2^- , which is generated at the first stage of autoxidation of D-glucosamine in the O_2 -containing aqueous solution, into $\cdot\text{OH}$ via H_2O_2 , and that $\cdot\text{OH}$ is finally responsible for the DNA strand-breakage. In addition to undergoing disproportionation, O_2^- is also known to react with H_2O_2 to give $\cdot\text{OH}$.²⁰⁾

Binding of Cu^{2+} to DNA

The effects of pFP6 linear DNA, which is distinguishable from ccc, oc- and linear DNA of pBR322 by agarose gel electrophoresis, on D-glucosamine-induced DNA strand-breakage in the presence of Cu^{2+} was then examined. As Fig. 2 shows, the reaction was inhibited by adding pFP6 linear DNA in a concentration-dependent manner. In the absence of Cu^{2+} , however, the reaction was not affected by the increasing concentration of added pFP6 linear

Fig. 3. Binding of Cu^{2+} to pBR322 ccc-DNA.

pBR322 ccc-DNA (100 μg) was incubated with various concentrations of CuCl_2 at 37°C in 10 mM Tris-HCl buffer (pH 8.0) for 3 hr, and the mixture (1 ml) were dialysed against 1000 ml of the same buffer overnight at 4°C with 3 changes of buffer to remove free CuCl_2 . The amounts of Cu bound to ccc-DNA were measured by atomic absorption analysis. 1 mM = 63.54 ppm.

Table V. EFFECTS OF DEOXYRIBONUCLEOTIDE ON THE STRAND BREAKAGE OF pBR322 ccc-DNA BY D-GLUCOSAMINE

pBR322 ccc-DNA was incubated with 0.1 M D-glucosamine at 37°C in 50 mM Tris-HCl buffer (pH 7.2) in the presence of each deoxyribonucleotide for 3 hr with or without 1 mM CuCl_2 . Percent inhibition of the DNA strand breakage by each addition was the same as that in the footnote of Table III.

Additions	Concn. (mM)	Inhibition (%)	
		$\text{Cu}^{2+}(-)$	$\text{Cu}^{2+}(+)$
None	—	0	0
d-GMP	1	-16	0
	10	-50	9
	50	-69	20
	100	-73	28
d-AMP	1	-10	-12
	100	-51	14
d-CMP	1	-17	0
	100	-66	-2
d-TMP	1	-10	-3
	100	-75	0

DNA. These results suggest that Cu^{2+} may bind to DNA. To confirm that binding of Cu^{2+} to pBR322 ccc-DNA occurred, the bound Cu^{2+} was measured by atomic absorption analysis. After incubating the DNA with various concentrations of Cu^{2+} , the mixtures were dialysed to remove free Cu^{2+} , and the Cu in the inner dialysate was measured. As Fig. 3 shows, the amounts of Cu increased in a concentration-dependent manner, confirming that Cu^{2+} was bound to the DNA. It was also shown that these Cu^{2+} -treated DNAs were broken by D-glucosamine much more readily than untreated DNA (data not shown here).

To identify the sites on ccc-DNA at which Cu^{2+} preferentially binds, the effects of some deoxyribonucleotides on the DNA strand breakage by D-glucosamine was examined. As Table V shows, in the absence of Cu^{2+} , the reaction was stimulated by all the deoxyribonucleotides tested. Deoxyriboses did not affect the reaction in the presence or absence of Cu^{2+} , but phosphate stimulated the reaction (data not presented in this paper). These re-

sults suggest that the stimulating effects of deoxyribonucleotides in the absence of Cu^{2+} may be due to their phosphate groups. On the other hand, in the presence of Cu^{2+} , d-GMP inhibited the reaction to some extent, but the other deoxyribonucleotides hardly affected the reaction. Therefore, the binding sites of Cu^{2+} may be deoxyguanylate regions of the DNA, as has been shown previously by Pezzano and Podo.¹⁰⁾

Discussion

In this study we have confirmed and extended our previous observation that the stimulating effect of Cu^{2+} on the D-glucosamine-induced pBR322 ccc-DNA strand breakage is due mainly to its stimulating effect on the generation of a series of active oxygen molecules, especially $\cdot\text{OH}$, in the aqueous solution of D-glucosamine. This kind of mechanism, suggested here for Cu^{2+} , is essentially similar to those reported already for L-ascorbic acid,¹¹⁾ triose reductone,¹¹⁾ mitomycin C,¹²⁾ D-isoglucosamine,¹³⁾ and D-fructose-6-phosphate.¹⁴⁾ The optimum concentration of Cu^{2+} (approximately 1 mM) for stimulation of $\cdot\text{OH}$ generation in the aqueous solution of D-glucosamine was the same as that for stimulation of the D-glucosamine-induced DNA strand breakage. At this Cu^{2+} concentration, the amounts of both O_2^- and H_2O_2 generated in D-glucosamine solutions were almost negligible. From our results and a proposed mechanism of the production of active oxygens,^{19,20)} O_2^- and H_2O_2 are considered to be the intermediates, and $\cdot\text{OH}$ is the active molecule involved in the DNA strand breakage. At Cu^{2+} concentrations higher than 1 mM, $\cdot\text{OH}$ may be changed to stable molecules that are less reactive with the DNA.

The possibility of involvement of the other active oxygens, such as $^1\text{O}_2$ and $\text{HO}_2\cdot$, in the reaction may be neglected, although their concentrations were not measured in these experiments. Merkel and Kearns¹⁵⁾ have shown that $^1\text{O}_2$ has an extremely short length of life in aqueous solution. Kobayashi¹⁶⁾ has shown

that the concentrations of $\text{HO}_2\cdot$ recognized in aqueous solution of pH 7–8 are 1/100–1/1000 as much as that of O_2^- . The possible production of glucosamine radicals, which may take part in the DNA strand breakage in the aqueous solution of D-glucosamine, is a subject for further investigation.

Cu^{2+} was found to bind to the ccc-DNA of pBR322 by measuring the bound Cu by atomic absorption analysis. Eichhorn *et al.*^{17,18)} have shown that, besides Cu^{2+} , many divalent cations such as Mg^{2+} , Mn^{2+} , Zn^{2+} , Ni^{2+} , Co^{2+} and Hg^{2+} can be bound to phosphate groups of DNA, and thereby change the conformation of the helical structure of DNA. However, these divalent cations do not affect the D-glucosamine-induced DNA strand breakage. This may be because the stimulating effects of these divalent cations on the generation of active oxygens in the aqueous solution of D-glucosamine are much less than those of Cu^{2+} .

The D-glucosamine-induced pBR322 ccc-DNA strand breakage in the presence of Cu^{2+} was inhibited by the addition of a different (pFP6 linear) DNA, but the generation of $\cdot\text{OH}$ from the aqueous solution of D-glucosamine was not. These results demonstrate that Cu^{2+} 's function in the autoxidation of D-glucosamine is attributable to the Cu^{2+} -DNA complex produced, as has been suggested, also, by Shinohara *et al.*,¹¹⁾ using L-ascorbic acid and triose reductone. The produced Cu^{2+} -DNA complex may oxidize D-glucosamine in the vicinities to generate $\cdot\text{OH}$, which is finally responsible for the DNA strand breakage. The inhibition of the D-glucosamine-induced DNA strand breakage in the presence of Cu^{2+} by the addition of a different DNA, therefore, might be due to competitive binding of Cu^{2+} to the added DNA; this would decrease the amount of Cu^{2+} available to bind to the target pBR322 DNA. The reason why the generation of $\cdot\text{OH}$ from the aqueous solution of D-glucosamine was not affected by the addition of the DNA may be that D-glucosamine can be oxidized to the same extent either by Cu^{2+} -DNA complex or Cu^{2+} . Besides Cu^{2+} -DNA

complex, however, we can not exclude the possibility of the formation of Cu^{2+} -D-glucosamine or DNA- Cu^{2+} -D-glucosamine complexes. These will be the subjects of later investigation.

The facts that D-glucosamine-induced DNA strand breakage in the presence of Cu^{2+} was inhibited only by d-GMP among the deoxyribonucleotides tested and that the generation of $\cdot\text{OH}$ from the aqueous solution of D-glucosamine in the presence of Cu^{2+} was not inhibited by d-GMP (data not presented in this paper) indicate that d-GMP- Cu^{2+} complexes will take part in the $\cdot\text{OH}$ -generation from aqueous solution of D-glucosamine as DNA- Cu^{2+} complex dose. Deoxyguanilate regions of the DNA may be possible binding sites for Cu^{2+} and/or preferred DNA damage sites. The nucleotide sequences in DNA subject to strand breakage induced by D-glucosamine in the presence of Cu^{2+} are currently being investigated.

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