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DNA Strand Scissions by Hydroxamic Acids-Copper(II) Ion under Aerobic Conditions

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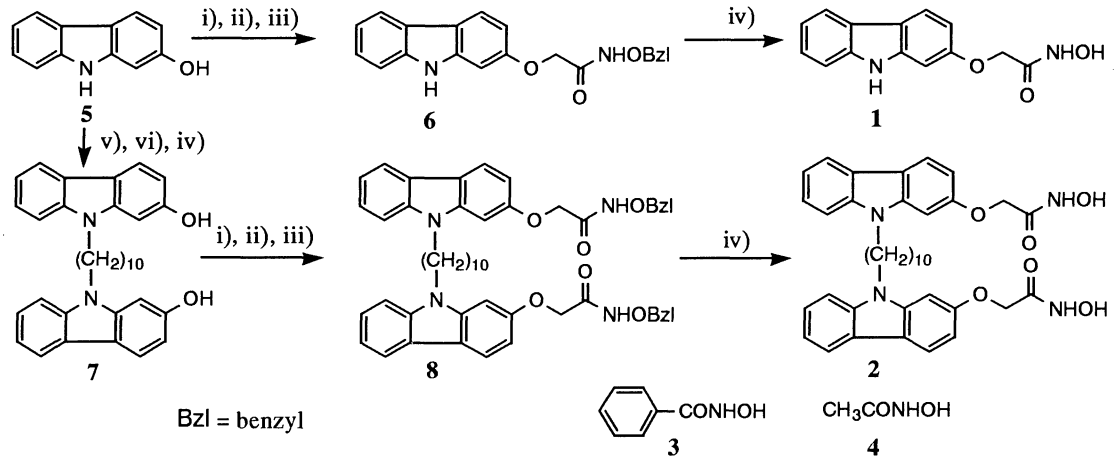
Carbazolyloxyacetohydroxamic acid (**1**), 9,9'-decamethylene-bis-carbazolyl-oxyacetohydroxamic acid (**2**), benzohydroxamic acid (**3**) and acetohydroxamic acid (**4**) without reducing agent under aerobic conditions induced Col E1 DNA strand scissions with increasing of the activities in the order of **4>3>2>1**. Inhibition experiments indicated that hydrogen peroxide and superoxide participated in the reactions, but hydroxyl radical or singlet oxygen did not.

Copper-complexes, such as 1,10-phenanthroline-Cu(II),[1] peptide-Cu(II)[2] or mitomycin-Cu(II),[3] with reducing agents under aerobic conditions induce DNA strand scissions mediated by reactive oxygen species (O_2^-, H_2O_2, $^1O_2$, ·OH). Certain hydroxamic acids are biologically a siderophore[4] and also show antitumor activity,[5] or chemically used as a metal-chelating agent[6] and a nucleophilic catalyst of hydrolytic enzyme models.[7] It is of interest how hydroxamic acids in the absence or presence of metal ion interact with DNA *in vitro* or *in vivo*. We now report DNA strand scissions by use of hydroxamic acids-Cu(II) without any reducing agent under aerobic conditions.

We designed carbazol-linked hydroxamic acid **1** and the bis-compound **2** expected to bind to DNA and to show a certain biological activity. The compounds **1** and **2** were synthesized according to the method shown in Scheme 1. The O-benzylhydroxamates **6** and **8** must be purified before the hydrogenation to give the pure **1** and **2** because the hydroxamic acids decompose during column chromatography for the purifications.



Scheme 1. Reagents: i) $BrCH_2COOCH_3$, K_2CO_3; ii) LiOH then HCl; iii) NH_2OBzl, DCC, DMF; iv) H_2/Pd-C; v) BzlCl, K_2CO_3; vi) $Br(CH_2)_{10}Br$, KOH, DMSO.

Cleavage of DNA was generally carried out under following conditions. Solutions of 0.3 μg Col E1 supercoiled closed circular DNA(form I) (in 1.5 μl Tris buffer), various concentrations of the hydroxamic acid (in 1.5 μl DMSO) and $CuCl_2$ (in 1.5 μl H_2O) in 40 mM Tris-HCl buffer (pH 8.0, total volume 15 μl) were incubated under aerobic conditions at 37 °C for 40 h. The DNA cleavages were followed by monitoring the conversion of the supercoiled DNA (form I) to the open circular form (form II). The results of the cleavages by **1-4** are shown in Table 1. The time course of the DNA cleavage by benzohydroxamic acid (**3**) in the presence of Cu(II) or by Cu(II) alone is shown in Fig. 1.

Table 1. Cleavage of Col E1 DNA by hydroxamic acids in the presence of Cu(II)[a)]

Hydroxamic acids	(mmol dm^{-3})	Cu(II) (mmol dm^{-3})	Form I (%)	Form II (%)
	---	---	84	16
	---	1.0	66	34
	---	0.5	65	35
	---	0.1	64	36
1	1.0	1.0	37	63
	0.5	0.5	59	41
	0.1	0.1	60	40
2	1.0	1.0	7	93
	0.5	0.5	23	77
	0.1	0.1	54	46
3	1.0	1.0	1	99
	0.1	0.1	5	95
4	0.1	0.1	0	100

a) Col E1 plasmid (form I) DNA(0.3 μg) in 40 mM Tris-HCl buffer (pH 8.0, 1.5 μl) was incubated with the hydroxamic acid (in 1.5 μl DMSO) and $CuCl_2$ (in 1.5 μl H_2O) in the Tris buffer (total volume 15 μl) under aerobic conditions at 37 °C for 40 h. The relative amounts of form I and form II were analyzed by 1% agarose gel electrophoresis and quantitated by fluorometric densitometry after ethidium bromide staining.

The results shown in Table 1 and Fig. 1 clearly indicate that the hydroxamic acids in the presence of Cu(II), which may exist as the hydroxamic acids-Cu(II) complexes *in situ*,[8)] without any reducing agent cause the DNA strand scissions. The DNA cleaving activities increased in the order of **4>3>2>1**. The order of the activities may be based on the steric factor and/or the DNA binding affinity (see below) of the substituents of the hydroxamic acids.

The displacements of ethidium bromide, bound to calf thymus DNA, with the hydroxamic acids **1-3** are shown in Fig. 2. These results show that in the absence of Cu(II), no DNA binding of **1** and **2**, as well as **3**, were observed. In contrast, in the presence of Cu(II), the DNA binding of the bis-carbazole **2** was

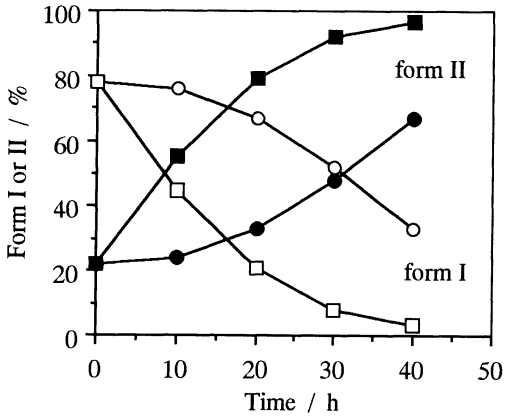


Fig. 1. Time course of DNA cleavages. The reactions were done under the same conditions as described in Table 1 except for time and concentration of reagents (each 0.1 mmol dm^{-3}). **3** + Cu(II): square; Cu(II) alone: circle.

remarkably increased and that of the mono-carbazol **1** slightly.[9] The increase in DNA binding by addition of Cu(II) results from complexing of the hydroxamic acid with Cu(II) *in situ*.[10] The differences of the DNA binding affinities of the mono-carbazol **1** and the bis-analog **2** in the presence of Cu(II) may correspond to those of the scission activities of them.

In order to examine whether the strand scissions are caused by hydrolysis of the phosphodiesters of DNA or radical reaction to decompose the sugar moiety, the reactions with inhibitors were carried out and the results are shown in Table 2. Catalase which removes H_2O_2 and Tiron which scavenges O_2^- effectively inhibited the DNA strand scissions. DABCO which scavenges $^1O_2$ slightly inhibited the scissions. SOD which decomposes O_2^- to H_2O_2 and O_2 did not inhibit the DNA cleavages. The results of inhibition experiments indicate that H_2O_2 and O_2^-provided from O_2 by the hydroxamic acids-Cu(II) complexes should play a very important role in the DNA strand scissions. In these reaction conditions, the hydroxamic acids should serve as a reducing agent, or electron donor, of Cu(II) to Cu(I), although the redox potential is not known yet.[10] Redox reaction of the hydroxamic acids-Cu(II) complexes may induce Cu(I) species which reacts with

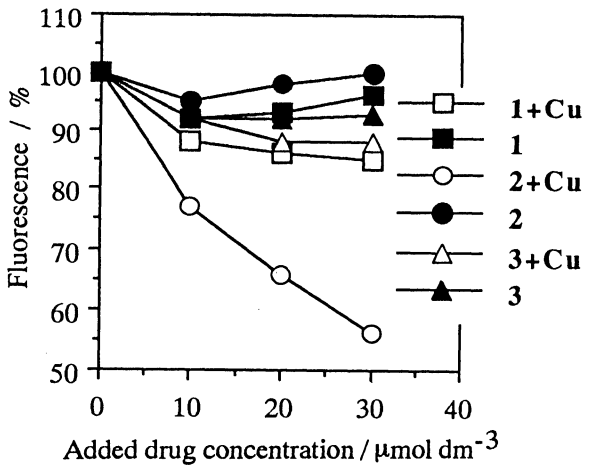


Fig. 2. Displacements of ethidium bromide (1.26 μmol dm^{-3}), bound to calf thymus DNA (1.0 μmol dm^{-3} bp), with **1-3** in the presence or absence of Cu(II). Fluorescence values are provided as percentages of the maximum.

Table 2. Inhibitions of the DNA cleavages by hydroxamic acids **2** and **3** in the presence of Cu(II) [a]

Hydroxamic acids (mmol dm^{-3})		Cu(II) (mmol dm^{-3})	Inhibitors		Form I (%)	Form II (%)
---		0.1		---	64	36
---			Catalase	10 (μg/ml)	81	19
---				1	81	19
2	1.0	1.0	---	---	7	93
			Catalase	100	78	22
				10	55	45
3	0.1	0.1	---	---	4	96
			Catalase	50	69	31
				10	55	45
				1	44	56
			SOD	100	2	98
				10	1	99
			Tiron	10 (mmol dm^{-3})	66	34
				5	58	42
			DABCO	10	13	87
				5	6	94

a) The reactions were done in the same conditions as described in Table 1 except for the inhibitors. SOD:superoxide dismutase; Tiron:4,5-dihydroxy-1,3-benzenedisulfonic acid; DABCO:1,4-diazabicyclo[2,2,2]octane.

O_2 to give O_2^- followed by H_2O_2 generation.[11] Hydroxyl radical and singlet oxygen could not participate in the DNA cleavages under these reaction conditions.[12] It has been reported that hydroxyl radical and singlet oxygen scavengers did not affected in the DNA cleavage by Cu(II)$(phen)_2$ with H_2O_2 and reducing agent.[13]

In conclusion, hydroxamic acids-copper complexes under aerobic conditions without any reducing agent induce DNA strand scissions due to the reactive oxygen species. This indicates that hydroxamic acids can be utilized as a new type of DNA scission agent in the presence of a metal ion.

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