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Different DNA Damaging Species as a Result of Oxidation of *n*-butyraldehyde and *iso*-butyraldehyde by Cu(II)

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The isomers *n*- and *iso*-butyraldehyde (BuA) in combination with Cu(II) induced single and double strand breaks in PM2 DNA, whereas the aldehydes, or Cu(II) alone had only negligible effect. The DNA damage was the result of radical oxidations of the aldehydes under formation of Cu(I). Cu(I) formation was independent of molecular oxygen. Extensive DNA degradation was only observed in the presence of molecular oxygen. Characterization of DNA damage pointed to different ultimate DNA damaging species. While catalase and neocuproine inhibited strand break formation induced by *iso*-BuA/Cu(II) to a high degree, these inhibitors were less effective in the *n*-BuA/Cu(II) reaction. On the other hand, sodium azide showed a high strand break inhibition in the *n*-BuA/Cu(II) reaction, but low inhibition in the *iso*-BuA/Cu(II) reaction. 2-Deoxyguanosine was hydroxylated in the 8-position by *iso*-BuA/Cu(II) but little reaction occurred with *n*-BuA/Cu(II). Chemiluminescence was detected during both BuA/Cu(II) reactions, whereby the intensity of the luminescence signal was 3.5-fold higher for *n*-BuA/Cu(II) than for *iso*-BuA/Cu(II). We suppose that the copper(II)-driven oxidation of *n*- and *iso*-BuA proceeds *via* different pathways with different DNA damaging consequences. Whereas the oxidation of *iso*-BuA mainly results in damage by •OH-radicals, the oxidation of *n*-BuA may lead to a radical reaction chain whereby excited states are involved and the resulting DNA-damaging species are not •OH-radicals.

Keywords: Aldehyde, copper, DNA damage, free radicals, excited carbonyl compounds

Abbreviations: BCS, bathocuproinedisulfonic acid; BSA, bovine serum albumin; BuA, butyraldehyde (butanal); dG, 2-deoxyguanosine; DMSO, dimethylsulfoxide; dsbs, double strand breaks; EDTA, ethylenediaminetetraacetic acid; HPLC-ECD, high performance liquid chromatography with electrochemical detection; 8-OHdG, 8-hydroxy-2-deoxyguanosine; PM2 DNA, supercoiled DNA from phage PM2; ROS, reactive oxygen species; SDS, sodium dodecyl sulphate; ssbs, single strand breaks; VaA, valeraldehyde (pentanal)

INTRODUCTION

Aldehydes represent a group of reactive organic compounds with widespread distribution as natural and synthetic products. Humans come in contact with aldehydes in various ways, for

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example as natural components of food or as additives in cosmetics or food products.[1] Endogenous formation of aldehydes by metabolic processes or from lipid peroxidation[2,3] are other sources of human exposure.

Recently, we have shown that aliphatic and aromatic aldehydes are oxidized by Cu(II) resulting in DNA strand breakage.[4] We proposed that the causative DNA damaging species are different in the aromatic and aliphatic aldehyde/Cu(II) reaction. During the Cu(II)-mediated oxidation of aromatic aldehydes the resulting Cu(I) is reoxidized by O_2 leading to $O_2^{\bullet -}$ and subsequently to hydrogen peroxide (Eqs. (1) and (2)):

$$Cu^{1+} + O_2 \rightarrow Cu^{2+} + O_2^{\bullet -} \quad (1)$$

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (2)$$

$$Cu^{1+} + H_2O_2 \rightarrow Cu^{2+} + OH^- + {}^{\bullet}OH \quad (3)$$

In these cases hydroxyl radicals formed during the Cu(I)-driven Fenton reaction (Eq. (3)) were found to be the causative DNA damaging molecules because strand breakage was completely suppressed by catalase and neocuproine. Similar mechanisms of DNA damage are also observed for redox reactions between Cu(II) and other organic molecules, like hydroquinones,[5,6] ascorbic acid,[7] or 2,4-dichlorophenoxyacetic acid,[8] and compounds with multiple oxygen functions, like tetracycline,[9] or flavonoids.[10]

For the aliphatic aldehydes, *n*-butyraldehyde/Cu(II) (*n*-BuA) and *n*-valeraldehyde/Cu(II) (*n*-VaA), a mechanism different from the described one leading to DNA strand breakage had to be proposed.[4] Here reactive oxygen species (ROS) probably play a minor role in DNA scission because of the ineffectivity of catalase and the weak effect of neocuproine in the prevention of DNA strand breaks.

The aim of this study was to characterize the reactive species responsible for DNA breakage by butyraldehyde/Cu(II). Beside the linear *n*-BuA also the branched isomer, *iso*-BuA, was included in the study.

MATERIALS AND METHODS

Chemicals and Reagents

Bromophenol blue, bovine serum albumin (BSA) and ethidium bromide were obtained from Serva (Germany), Seakem agarose was purchased from FMC, Rockland (USA). Neocuproine was from Fluka (CH), *n*-butyraldehyde, *iso*-butyraldehyde and $CuCl_2$ from Riedel de Haen (Germany). The aldehydes were stored under nitrogen. Purity of the aldehydes was determined according to their refraction index. Bathocuproine disulfonic acid and Brij-35 were obtained from Sigma (Germany), catalase (specific activity: 65 000 U/mg) was from Boehringer (Germany), 8-hydroxy-2-deoxyguanosine standard from Wako Chemicals (Germany), 9,10-dibromanthracene-2-sulfonate from Lancaster (UK) and sodium azide from Aldrich Chemie (Germany). Supercoiled DNA from the phage PM2 was produced and isolated in our laboratory according to the method of Espejo and Canelo.[11]

Chemical Treatment of PM2 DNA and Determination of DNA Strand Breaks

All solutions were freshly prepared before use. PM2 DNA (0.2 μg) was incubated with $CuCl_2$ (0.1 mM final concentration in experiments with 100 mM sodium phosphate buffer, pH 7.25, or 0.75 mM final concentration in experiments with 10 mM Tris · HCl pH 7.2) and butyraldehyde in various concentrations for up to 60 min at 37°C. For inhibition of single strand breaks catalase (10 or 70 μg/ml), DMSO (12 mM), neocuproine (0.4 mM) or sodium azide (10 mM) were added to the incubation mixture. The reactions were terminated by chilling the reaction mixture on ice and adding a solution containing 10% SDS, 10% Ficoll, 25% DMSO and 0.04% bromophenol blue in bidistilled water.

To separate the circular supercoiled, circular relaxed and linear PM2 DNA forms, horizontal agarose gel electrophoresis was carried out. Gels

were formed using 0.5% Seakem agarose dissolved in electrophoresis buffer (89 mM Tris · HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.4). Electrophoresis was run at a constant voltage of 2.7 V/cm for 3.5 h. The gels were stained with ethidium bromide (1 mg/l in water) for 1 h in the dark and then treated with 1 mM $MgSO_4$ for 15 min to remove excess ethidium bromide. Gels were illuminated from below with UV light (Biometra fluo-link TFL-20.M, emitting predominantly at 312 nm) and photographed on Polaroid type 667 films. The intensities of different PM2 DNA forms were measured directly from the photographs with a Chromoscan scanning densitometer (Joyce & Loebl Ltd., England). Peaks were then integrated and the number of single strand breaks per PM2 DNA molecule were calculated as described by Buschfort and Witte.[9]

Determination of Cu(I)

Cu(I) generation was determined by using the Cu(I) reagent bathocuproine disulfonic acid (BCS) according to the method of Rahman *et al.*[12] Butyraldehyde (10 mM) was mixed with $CuCl_2$ (10 μM) and BCS solution (0.4 mM) in sodium phosphate buffer (100 mM, pH 7.25) and incubated at 37°C. The stable BCS–Cu(I) complex was determined by measuring its absorbance at 480 nm. To calculate the aldehyde-specific formation of Cu(I) the reduction of Cu(II) in the BCS solution was subtracted.

Hydroxylation of 2-deoxyguanosine by the Mixture of Butyraldehyde/$CuCl_2$ and Detection of 8-hydroxy-2-deoxyguanosine by HPLC-ECD

The formation and the detection of the hydroxylation product 8-hydroxy-2-deoxyguanosine (8-OHdG) of 2-deoxyguanosine (dG) was done according to the methods of Floyd *et al.*[13] and Haegele *et al.*[14] dG (2 mM) was treated with *iso*- or *n*-BuA (0.6–30 mM) alone or in combination with $CuCl_2$ (0.1 mM) in sodium phosphate buffer (100 mM, pH 7.25) for 10 min, 1 and 3 h at 37°C under aerobic conditions. The HPLC system consisted of a Waters millipore® model 510 pump, Waters millipore® U6K injector, Waters millipore® model 460 electrochemical detector (ECD) and a reversed-phase Waters Delta Pak (5 μ, C18, 300 Å, 3.9 × 150 mm) column. The mobile phase consisted of potassium phosphate buffer (50 mM, pH 5) containing methanol (4.5%, v/v) and the injection volume was 20 μl. The flow rate was 1 ml/min and the voltage of the glassy-carbon working electrode was set to +0.7 V.

Measurement of Chemiluminescence in the Mixtures of Butyraldehyde/$CuCl_2$

Chemiluminescence was examined in a standard single photon counting setup equipped with a red sensitive photomultiplier. According to the method of Brunetti *et al.*[15] a mixture of Brij-35 (final concentration 3 mM), 9,10-dibromoanthracene-2-sulfonate (final concentration 10 μM) and ethanol (final concentration 40 μM) was dissolved in sodium phosphate buffer (100 mM, pH 7.25). A stock solution of butyraldehyde (final concentration 50 mM) was added and the level of chemiluminescence was detected for about 4 min. After addition of $CuCl_2$ (final concentration 0.1 mM) the detected photons were integrated over 1 s and calculated as a function of the reaction time.

RESULTS

Induction of DNA Strand Break Formation

Both isomers *n*-BuA and *iso*-BuA induced DNA single (ssbs) and double strand breaks (dsbs) in combination with $CuCl_2$. As seen in Figure 1, DNA dsb formation by *n*-BuA/$CuCl_2$ was more pronounced than by *iso*-BuA/$CuCl_2$. At 25 mM *n*-BuA/0.1 mM $CuCl_2$ nearly all DNA molecules contained more than two dsbs which is shown by

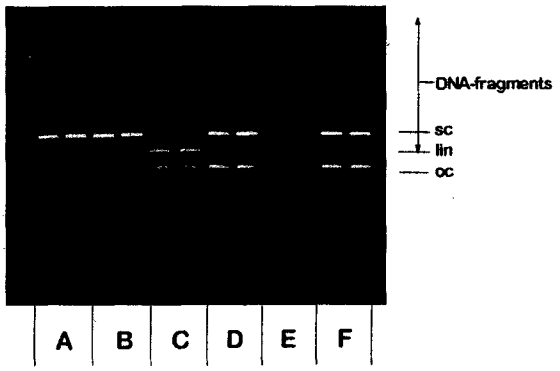


FIGURE 1 Agarose gel stained with ethidium bromide. PM2 DNA was incubated with A: *iso*-butanal (25 mM); B: *n*-butanal (25 mM); C: *iso*-butanal (25 mM) and $CuCl_2$ (0.1 mM); D: *iso*-butanal (0.5 mM) and $CuCl_2$ (0.1 mM); E: *n*-butanal (25 mM) and $CuCl_2$ (0.1 mM); F: *n*-butanal (0.5 mM) and $CuCl_2$ (0.1 mM). The reaction mixtures were incubated for 1 h at 37°C in sodium phosphate buffer (100 mM, pH 7.25). The positions of supercoiled (sc), linear (lin), open circular relaxed (oc) DNA as well as DNA fragments are indicated.

TABLE I Inhibition of DNA single strand break formation by catalase, DMSO, neocuproine or sodium azide. The aldehydes (1 mM) were incubated for 1 h at 37°C with $CuCl_2$ (0.1 mM) and additives in sodium phosphate buffer (100 mM, pH 7.25). Experiments were performed twice, each with three parallel samples. The standard deviation was about 12%

Additive	Inhibition (%)	
	iso-butanal	*n*-butanal
Catalase (65 000 U/mg)		
10 μg/ml	82	23
70 μg/ml	81	19
Catalase inactivated (5 min, 100°C)		
10 μg/ml	0	5
DMSO		
12 mM	42	12
Neocuproine		
0.4 mM	45	25
Sodium azide		
10 mM	7	62

a smear and the absence of distinct DNA bands in the gel. At the same concentrations *iso*-BuA/$CuCl_2$ induced ssbs only in half of the DNA molecules and one dsb in the other half.

To characterize the strand break formation catalase, DMSO, neocuproine, or sodium azide were added to the incubation mixture containing DNA, BuA and $CuCl_2$. Catalase indicates whether or not hydrogen peroxide is involved which, if it is, suggests the involvement of •OH-radicals from the Cu(I)-driven Fenton reaction in strand breakage. Additionally DMSO was added as an •OH-radical quencher. The role of Cu(I) in strand break formation was tested by adding neocuproine, which is able to complex Cu(I) in a redox inactive form. Inhibitory effects of sodium azide point to excited species, like singlet oxygen[16] or excited triplet molecules, involved in strand break induction. As shown in Table I, the effects of the additives on DNA damage were different in the *n*-BuA/$CuCl_2$ and the *iso*-BuA/$CuCl_2$ mixtures. The addition of catalase (10 μg/ml) to the *iso*-BuA/$CuCl_2$ reaction inhibited strand break formation more than 80%, the inhibitory effect in the *n*-BuA/$CuCl_2$ experiments was about 20%. The enzyme itself was not inhibited in the reaction mixture. This is shown by comparable strand break inhibition effects of at 10 and 70 μg/ml (Table I). Similar to catalase, DMSO also inhibited DNA strand breakage induced by *iso*-BuA/$CuCl_2$ more effectively than by *n*-BuA/$CuCl_2$. Neocuproine suppressed strand breakage incompletely in both reactions, in the *iso*-BuA/$CuCl_2$ reaction by 45% and in the *n*-BuA/$CuCl_2$ reaction by 25%. While sodium azide had a negligible effect on the *iso*-BuA/$CuCl_2$ reaction, experiments with *n*-BuA/$CuCl_2$ showed an inhibitory effect of 62%.

To evaluate the role of oxygen in strand break formation the experiments were performed under aerobic and anaerobic conditions. Figure 2 shows that DNA strand breaks induced either by *n*-BuA/$CuCl_2$, or by *iso*-BuA/$CuCl_2$ are suppressed in the absence of oxygen.

Formation of Cu(I) during the Butyraldehyde/$CuCl_2$ Reaction

The BuA/$CuCl_2$ reaction was performed under aerobic and anaerobic conditions. As seen in

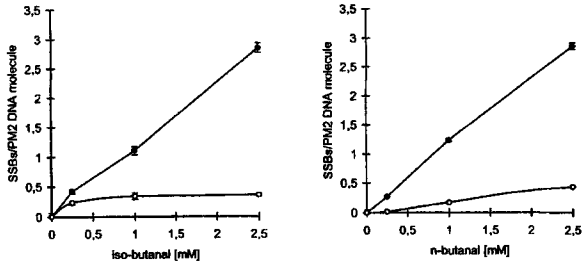


FIGURE 2 Single strand break induction in PM2 DNA after 1 h incubation under aerobic (closed symbols) and anaerobic (open symbols) conditions. The aldehydes were incubated with $CuCl_2$ (0.75 mM) for 1 h at 37°C in Tris · HCl (10 mM, pH 7.25).

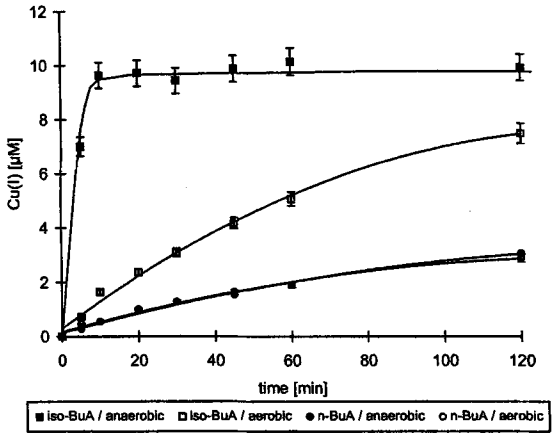


FIGURE 3 Cu(I) production measured as Cu(I)-bathocuproine disulfonic acid (BCS) complex in aldehyde/$CuCl_2$ reaction mixtures with 0.4 mM BCS. The aldehyde concentration was 10 mM, the $CuCl_2$ concentration was 10 µM. Incubation was carried out for 1 h at 37°C in sodium phosphate buffer (100 mM, pH 7.25).

Figure 3 the Cu(I)-bathocuproine complex is formed during the *iso*- and *n*-BuA/$CuCl_2$ reactions under aerobic conditions. Thereby the Cu(I) concentration increased continuously with time. After two hours of incubation 70% and 25% of Cu(II) was reduced in the *iso*-BuA and the *n*-BuA mixtures, respectively.

If *n*-BuA/$CuCl_2$ was reacted under argon, the same formation rate of the Cu(I)-bathocuproine complex was observed as in the presence of oxygen. In contrast, the initial rate of Cu(I) formation in the *iso*-BuA/$CuCl_2$ reaction was

TABLE II Formation of 8-hydroxy-2-deoxyguanosine (8-OHdG). The reaction mixtures were composed of 2-deoxyguanosine (2 mM), *iso*- or *n*-butanal in different concentrations and $CuCl_2$ (0.1 mM). Samples were incubated in sodium phosphate buffer (100 mM, pH 7.25). The detection limit was 3.7 ng/ml, the standard deviation about 10%

		8-OHdG (ng/ml)		
		10 min	1 h	3 h
iso-butanal	0.6 mM	15.2	27.9	30.7
	6 mM	46.1	151.3	219.0
	30 mM	160.8	470.9	674.2
	30 mM*	27.5	34.1	35.3
n-butanal	0.6 mM	13.2	10.6	13.3
	6 mM	6.0	12.0	11.3
	30 mM	9.4	16.8	16.6
	30 mM*	9.5	9.0	6.9

*Experiments without $CuCl_2$ in the reaction mixture.

enhanced under anaerobic conditions (Figure 3). Within 10 min nearly 100% of the Cu(II) added was reduced redoxinactively complexed as Cu(I), as compared to 15% in the presence of O_2.

Formation of 8-hydroxydeoxyguanosine (8-OHdG)

The formation of 8-OHdG points to DNA damage by •OH-radicals[17,18] or $^1O_2$, respectively.[19] To examine the role of these oxygen species in DNA breakage induced by BuA/$CuCl_2$, 2-deoxyguanosine (dG) was incubated with *iso*- or *n*-BuA and $CuCl_2$ under physiological conditions. Thereafter 8-OHdG formation was determined by HPLC-ECD. As seen in Table II, dG was hydroxylated by *iso*-BuA/$CuCl_2$ in a concentration and time dependent manner, whereas *n*-BuA/$CuCl_2$ produced only traces of 8-OHdG under the same conditions.

Formation of Excited Species

The inhibitory effect of sodium azide in the strand break induction by *n*-BuA/$CuCl_2$ pointed to the participation of excited species in DNA damage. Both, the *n*-BuA/$CuCl_2$ reaction and the *iso*-BuA/$CuCl_2$ reaction produced a chemiluminescence

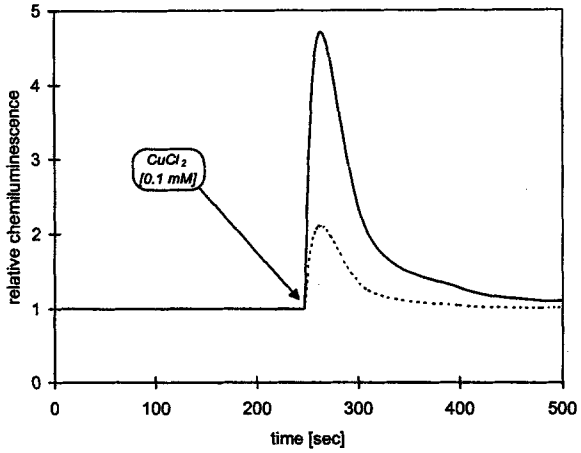


FIGURE 4 Comparison of the chemiluminescence intensity of the reaction mixtures *iso*-butanal (···) (50 mM) or *n*-butanal (—) (50 mM) after the addition of $CuCl_2$.

signal (Figure 4). The intensity of this signal was 3.5-fold higher for *n*-BuA/$CuCl_2$ than for *iso*-BuA/$CuCl_2$.

DISCUSSION

We demonstrated that *n*-BuA and *iso*-BuA were oxidized under physiological conditions by Cu(II) resulting in DNA single and double strand breaks. Formation of ssbs was similar in both reactions. Double strand breakage as the result of two single strand breaks in site-specific DNA damage[4] was more pronounced in the *n*-BuA/Cu(II) reaction than in the *iso*-BuA/Cu(II) reaction. In both reactions DNA strand breaks were drastically reduced in the absence of oxygen.

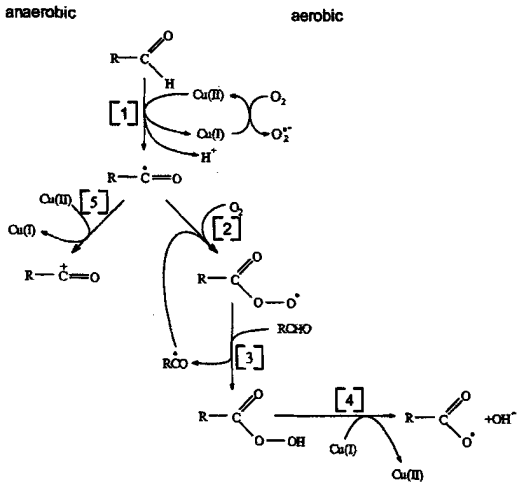
In spite of the similar DNA strand breaking properties of the *n*-BuA and *iso*-BuA/Cu(II) reactions, significant differences were observed in the formation of 8-OHdG and excited states. In addition the effects of catalase, DMSO, neocuproine and sodium azide on DNA damage differed. This points to different reaction mechanisms of *n*-BuA and *iso*-BuA in combination with Cu(II).

For *iso*-BuA/Cu(II) the results indicated the formation of ROS during the redox reaction. The reduction of DNA strand breaks by 83% under anaerobic conditions as well as the inhibitory effects of catalase, DMSO and neocuproine in strand breakage leads to the conclusion that •OH-radicals are the causative DNA damaging species produced during the Cu(I)-driven Fenton reaction. The low inhibitory effect of neocuproine (45%), DMSO (42%) and catalase (83%) indicates that •OH-radicals from the Cu(I)-driven Fenton reaction are not the only DNA strand breaking species. Hydroxylation of deoxyguanosine in the 8-position by *iso*-BuA/Cu(II) confirm that •OH-radicals were formed and are responsible for DNA damage. Because sodium azide, a $^1O_2$ scavanger, did not prevent DNA scission, it is unlikely that $^1O_2$ was involved in the generation of 8-OHdG.

The presence of oxygen during the *iso*-BuA/Cu(II) reaction is supposed to be a basic requirement for strand breakage but not for Cu(I) formation. Cu(I) was formed in the presence as well as in the absence of oxygen. This means the first step of the *iso*-BuA oxidation by Cu(II) is independent of oxygen but not the reaction directly prior to DNA strand breakage. Some aldehydes are known to undergo redox reactions with metal ions or enzymes. Although the different steps of these interactions are not proven in this study, they are suitable to support our results.

As a primary intermediate of the metal catalyzed aldehyde oxidation the acyl radical was proposed as shown in Scheme 1, step 1.[20,21] Bawn *et al.*[21] described subsequent reactions of the acyl radical under nonphysiological conditions. The addition of molecular oxygen to the acyl radical leads to the formation of the peroxyacid radical (Scheme 1, step 2). By abstraction of an •H-radical from another aldehyde the peroxyacid radical is transformed into peroxyacid and a new acyl radical. This reaction (Scheme 1, step 3)[21] enables the formation of the acyl radical in the absence of copper. Further radical decomposition of the peroxyacid is facilitated in the presence of Cu(I) (Scheme 1, step 4).[22,23]

In the absence of molecular oxygen an enhanced Cu(I) formation rate was observed.



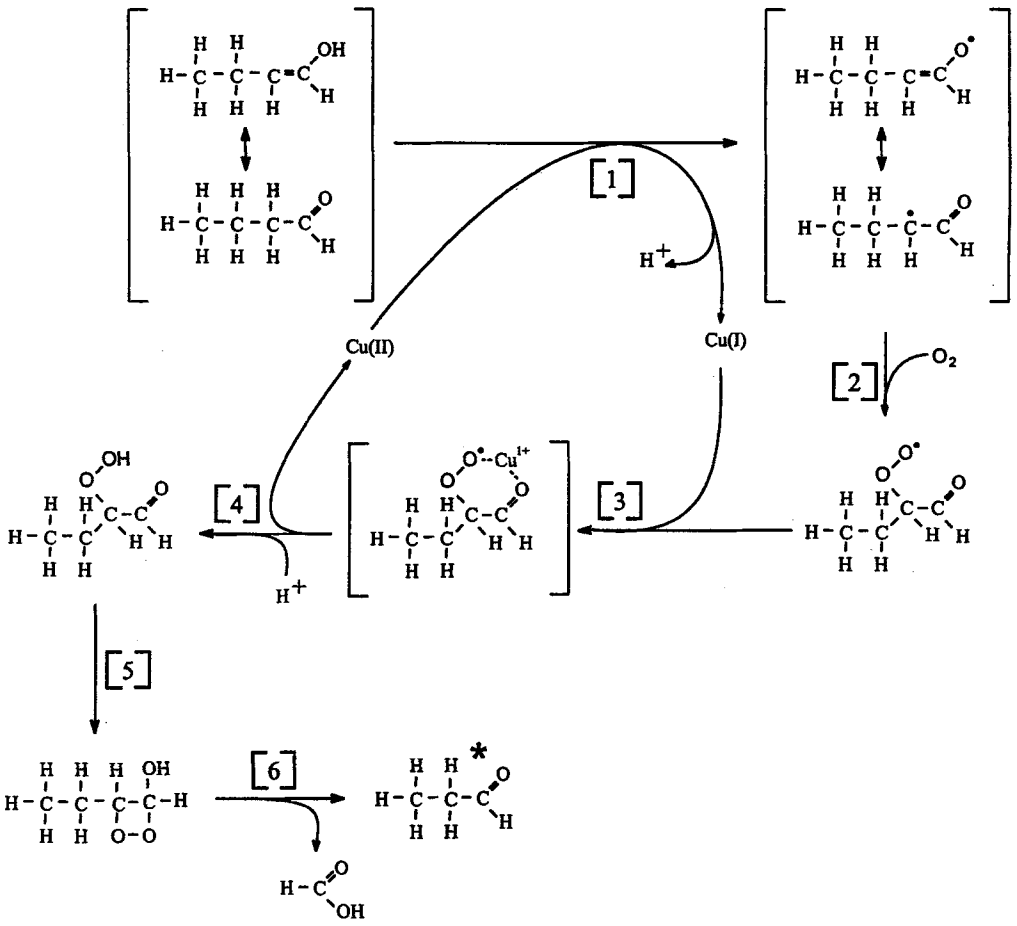
SCHEME 1 Proposed reaction scheme for the interactions of *iso*-butanal and Cu(II) under aerobic and anaerobic conditions. For references see the text.

This points to different pathways of the Cu(II) mediated oxidation of *iso*-BuA under aerobic and anaerobic conditions. After the first oxygen independent initiation step (Scheme 1, step 1) the resulting acyl radical may be further oxidized under anaerobic conditions by another Cu(II) ion leading to the carbenium ion (Scheme 1, step 5). This reaction has been demonstrated for acetaldehyde under aerobic conditions whereby Cu(II) competes successfully with O_2.[23] The oxidation mechanism of two one-electron transfers (Scheme 1, steps 1 and 5) is in agreement with the observed enhancement of Cu(I) formation during the *iso*-BuA/Cu(II) reaction in the absence of oxygen.

While in the presence of oxygen •OH-radicals from the Cu(I)-driven Fenton reaction (Eq. (3)) are the main candidates for DNA strand breakage, strand breaks detected under anaerobic conditions may be the consequence of DNA alkylation by carbenium ions. Carbenium ions formed under anaerobic conditions (Scheme 1, step 5) are reactive electrophilic species known to attack DNA bases.[24,25] The DNA adducts formed lead to apurinic/apyrimidinic (AP) sites and subsequently at least in part to single strand breaks.[26] In addition, intermediates like C-centered acyl radicals and peroxyl radicals (Scheme 1, steps 1–3), which are described as DNA reactive,[27,28] can also lead to DNA adducts, AP-sites and strand breaks. These strand breaks cannot be prevented by inhibitors like catalase or neocuproine and may explain their incomplete inhibition in the *iso*-BuA/Cu(II) reaction.

Strand break formation in the *n*-BuA/Cu(II) reaction proceeded at a greater rate under aerobic conditions than in the absence of O_2. This means, that O_2 is a participant in the reaction. The low inhibitory effect of catalase (about 20%) and DMSO (12%), the negligible formation of 8-OHdG and the minor role of neocuproine in preventing DNA strand breaks, all exclude •OH-radicals as the main causative agents of DNA scission. A chemiluminescence signal was detected, which suggests to the formation of an excited intermediate during the *n*-BuA/Cu(II) reaction. Also, the strong inhibitory effect of sodium azide in DNA strand breakage by *n*-BuA/Cu(II) points to DNA damage induced by excited species or subsequent reactions of these species. We assume that $^1O_2$ is not the ultimate strand breaking agent, because in combination with the low formation rate of 8-OHdG the DNA damaging profile induced by *n*-BuA/Cu(II) differs from that of $^1O_2$, where low strand breakage and high 8-OHdG formation rates are generally observed.[29,30]

The formation of excited triplet states during aldehyde oxidation has been described in a reaction catalyzed by horse raddish peroxidase (HRP).[31,32] We suppose that Cu(II) can mimetice the initiation reaction of HRP. Thereby an α-C-centered radical (Scheme 2, step 1) and subsequently, after addition of molecular oxygen (Scheme 2, step 2), an α-peroxyl radical is formed. Subsequent reactions are leading to an unstable 1,2-dioxetane ring (Scheme 2, steps 4 and 5), that decomposes to formic acid and an excited triplet carbonyl compound (Scheme 2, step 6), which can be detected by chemiluminescence measurement. Cu(I) formed during the *n*-BuA/Cu(II) reaction presumably does not reduce molecular oxygen because we found catalase



SCHEME 2 Proposed reaction scheme for the interactions of *n*-butanal and Cu(II). For references see the text.

and neocuproine showing only negligible effects on DNA strand break inhibition. Complexation of Cu(I) by the α-peroxy and the aldehyde oxygen (Scheme 2, step 3) should facilitate the reduction of the peroxyl radical and favor it over the reduction of O_2 by Cu(I).

Based on this reaction course it also becomes obvious why aliphatic unbranched aldehydes become more reactive in combination with Cu(II) when the chain length increases. This was shown with *n*-BuA and *n*-valeraldehyde (*n*-VaA (pentanal)) in a previous study.[4] If both aldehydes were oxidized according to the HRP reaction the resulting products should be a new aldehyde shortened by one C-atom. In the case of *n*-VaA the product *n*-BuA is expected. This newly formed aldehyde should be able to perform an additional reaction cycle, one redox cycle more by *n*-VaA/Cu(II) than by *n*-BuA/Cu(II). The lower number of strand breaks observed for *n*-BuA/Cu(II) compared to *n*-VaA/Cu(II) is in agreement with the postulated reactions.

In conclusion, the oxidation of aliphatic aldehydes by Cu(II) may initially occur at the carbonyl C-atom or at the α-C-atom with different DNA damaging consequences. Oxidation of the carbonyl C-atom results mainly in DNA damage by $^{\bullet}$OH-radicals, oxidation of the α-C-atom leads to a radical reaction chain where excited triplet states are involved and DNA damaging species

are different from •OH-radicals. It should be noted that neither the *n*-BuA/Cu(II) nor the *iso*-BuA/Cu(II) reaction followed exclusively one reaction pathway. The minor pathway for *iso*-BuA/Cu(II) is demonstrated by the low chemiluminescence signal and for the *n*-BuA/Cu(II) reaction by the low inhibitory effect of catalase and neocuproine as well as the minor formation of 8-OHdG.

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