Genotoxicity of Halogenated Propenals, Environmentally Relevant Oxygen Containing Short Chain Halocarbons

Erwin Eder, Elisabeth Weinfurtner and Matthias M.G. Eder
Institute of Toxicology, University of Würzburg D-97078 Würzburg,
Versbacher Str. 9, Germany

Introduction

2-Halogensubstituted acroleins and its congeners e.g. 2-chlorocinnamaldehyde, 2-bromocinnamaldehyde or 2-chlorocrotonaldehyde are very potent mutagens in Salmonella typhimurium strain TA 100[1]; the 2-haloacroleins are also carcinogens[2]. Base pair substitutions are considered to be the genotoxic mechanism underlying the mutagenic and carcinogenic activities of these compounds[1]. We investigated mutagenicity in several Salmonella strains, genotoxicity in the SOS-chromotest as well as formation of DNA-adducts and established structure activity relationships in order to obtain better insights into the genotoxic mechanisms of this type of compounds[3].

Formation and environmental occurrence

Chlorosubstituted alkenals are formed and released by chlorination of organic matter e.g. during chlorine bleaching of paper pulps or chlorination of drinking water containing natural humic acids[4], and bromosubstituted alkenals are formed during chlorination in the presence of bromine e.g. mineral bromides. These compounds are also secondary products of several pesticides e.g. certain chloroallylthiocarbamates or dichloropropenes and are directly utilized in agriculture[4,5].

Material and Methods

The test substances were either synthesized in our laboratory and characterized by ^{1}H-NMR, ^{13}C-NMR, FT-IR, UV, mass spectroscopy and melting points, boiling points, elemental analysis etc. or purchased in the highest purity available. Purities were checked by capillary gas chromatography or HPLC and identity by ^{1}H-NMR or by GC-MS. In most cases the bought compounds were additionally purified immediately before use (recrystallization, distillation, preparative GC or HPLC). Purities were at least 98%.

Mutagenicity and Genotoxicity
The Ames preincubation mutagenicity test[6] or the liquid test[7] were performed with *different Salmonella typhimurium* strains. The SOS-chromotest according to Quillardet and Hofnung[8] was carried out with the Escherichia coli strain PQ 37.

DNA-binding studies
DNA-adducts were synthesized by reaction of different alkenals with nucleosides, calf thymus DNA, bacterial suspension or with liver-DNA using the isolated perfused liver technique[9]. The adducts were isolated by comprehensive chromatographic methods and characterized by ^{1}H-NMR, ^{13}C-NMR, FT-IR, UV and mass spectrometry as well as by melting points and elemental analysis. The chromatographic properties were determined.

Results

Genotoxic activities

The results are shown in Table 1

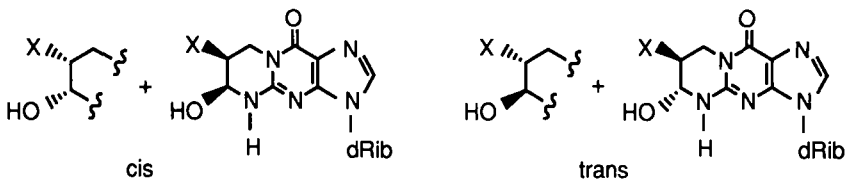
Table 1 Genotoxic Activities of Halosubstituted Enals

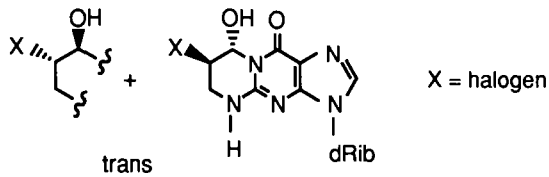
Substance	Mutagenicity (Revertants/µmol) (without S9 mix)					SOS-chromotest	
	strain TA 100	TA 98	TA 1535	TA 1538	his D 3052	SOSIP	I_{max}
2 CA	1,325,300	nd	233,333	nd	nd	13,750 x 10^{-3}	5.67
2 BA	730,820	nd	173,611	nd	nd	25,200 x 10^{-3}	7.54
2 CCA	208,333	nd	nd	nd	nd	5,900 x 10^{-3}	9.39
3 CCA	0	0	0	0	184	3.5 x 10^{-3}	1.63
2 C 33 DA	43,243	0	nd	0	nd	79 x 10^{-3}	9.87
2 CC	6,081	3,050	nd	nd	nd	18 x 10^{-3}	6.91
2 BC	105,500	41,567	2,110	15,825	nd	150 x 10^{-3}	19.55

nd = not determined
2 CA = 2-Chloroacrolein
2 BA = 2-Bromoacrolein
2 CCA = 2-Chlorocrotonaldehyde
3 CCA = 3-Chlorocrotonaldehyde
2 C 33 DA = 2-Chloro,3,3-dimethylacrolein
2 CC = 2-Chlorocinnamaldehyde
2 BC = 2-Bromocinnamaldehyde

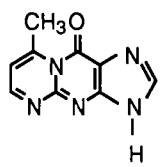
All tested compounds except 3-chlorocrotonaldehyde were clearly mutagenic in Salmonella typhimurium strain TA 100. The tested 2-haloacroleins and 2-bromocinnamaldehyde were also mutagenic in strain TA 1535 not containing the pkM 101 plasmid. The halocinnamaldehydes were also positive in the frameshift sensitive strain TA 98 and bromocinnamaldehyde also in the strain TA 1538 not containing the pkM 101 plasmid.
3-Chlorocrotonaldehyde is positive only in the frameshift sensitive strain his D 3052 which does not contain the rfa and the UVrB mutation i.e. this strain possess still an intact cell wall and it is still capable of performing excision repair. All tested substances induce SOS repair in the SOS-chromotest.

Adduct formation
With both 2-haloacroleins we isolated two different regioisomers of cyclic 1,N^2-propanodeoxyguanosine adducts:





A pair of diastereomers of cis isomers (traces) and a pair of diastereomers of the trans-isomers of regioisomer type A were found whereas in the case of the regioisomer type B only a pair of diastereomers of the trans-isomer was found.
In the case of the 3-methylsubstituted 2-chloroacrolein congeners only the diastereomers of the type B regioisomers were identified. No adduct formation was observed with the halocinnamaldehydes.
A completely different type of cyclic 1,N^2-guanine adducts was formed by 3-chlorocrotonaldehyde:



Evidently, H_2O and HCl are eliminated and an unsaturated conjugated system results.

Structure activity relationship
Both, mutagenicity in the Ames test and genotoxicity of the 2-haloenals in the the SOS-chromotest decrease with increasing degree of substitution. Furthermore, reactivity of the 2-chloroalkenals towards deoxyguanosine also decreases with increasing degree of substitution.

Discussion and Conclusions; Genotoxic Mechanisms and Risk Assessment

The 2-haloacroleins and its methylsubstituted congeners induce base pair substitution and lead to SOS repair. Mutagenicity is clearly higher in the base pair sensitive strain TA 100 capable of performing error prone repair (SOS repair) via the pkM 101 plasmid than in strain TA 1535 not containing this plasmid; the latter strain is, however, otherwise

identical with TA 100. Furthermore mutagenic and genotoxic activities of these compounds run in parallel with their reactivity towards deoxyguanosine[3,10]. The 2-halocinnamaldehyde show high mutagenicity in the frame shift sensitive strains TA 98 and TA 1538 most probably due to their planar structures. Evidently error prone repair is also induced because mutagenicity in strain TA 98 is higher than in TA 1538 not containing the pkM 101 plasmid and because SOS repair is induced in the SOS-chromotest.
3-Chlorocrotonaldehyde also induces frame shift in strain his D 3052. Evidently this strain is less sensitive for toxic effects than the respective strains TA 1538 and TA 98 which contain the rfa and the UVrB mutation.
In general, the high bacterial toxicity of haloalkenals interferes with the bacterial mutagenicity and genotoxicity testing and it may well be that mutagenic activities are actually even higher. Higher doses which could lead to higher mutagenicities cannot be applied due to the toxicity.
The tested haloenals were shown to be very potent mutagens and genotoxins interacting with DNA-components and some revealed to be clear carcinogens. These compounds are released to the environment and are found in drinking water and surface water. It may well be that the mutagenic and carcinogenic risk from these substances have been underestimated to date.

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