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FORMATION OF BURST CHEMILUMINESCENCE, EXCITED ALDEHYDES, AND SINGLET OXYGEN IN MODEL REACTIONS AND FROM CARCINOGENIC COMPOUNDS IN RAT LIVER S9 FRACTIONS

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Abstract

It is shown that in physiological circumstances (T=298 K, pH=7.4) various aldehydes (formaldehyde, acetaldehyde, glyoxal, methyl-glyoxal, etc.) can be activated by hydrogen peroxide (H_2O_2) in the presence of the ϵ-amino group of L-lysine, with simultaneous formation of singlet oxygen ($^1O_2$) and chemiluminescence. The activated aldehydes are in an excited state and have radical structure causing extremely high reactivity and a possible attack of cellular components like proteins, amino acids, RNA and DNA. In the reactions methylated, formylated, acetylated, etc. compounds are formed as well. Activated aldehydes can also be formed during the biological oxidation of different nitrosamines (DMN, DEN, N-nitroso-morpholine) and formaldehyde-hydrazine adduct. It is proved that in rat liver S9 fractions these activated (excited) aldehydes, especially formaldehyde, have been formed with a simultaneous burst chemiluminescence emission and appearance of singlet oxygen. In rat liver S9 fractions excited formaldehyde can be liberated only after 30 min preincubation time from dimethyl-nitrosamine (DMN) and monomethyl-hydrazine (MMH). On the other hand, excited formaldehyde is liberated immediately from hydrazine-formaldehyde-adduct (HZ/FORM) and isonicotinic acid-hydrazide-formaldehyde adduct (INH/FORM), i.e. no preincubation is necessary.

Keywords: chemiluminescence, excited aldehydes, singlet oxygen, S9 rat liver fractions, carcinogenesis.

Introduction

The metabolism of different N-nitrosamines (DMN, DEN, N-nitrosomorpholine, etc.) by rat microsomes results formaldehyde, acetaldehyde, glyoxal, etc. [1–3]. These oxidations can take place with the help of cytochrome P–450 monooxygenase systems. However, some details of these processes are still not clear. Questions to be answered are (i) how these aldehydes are formed in the metabolism, (ii) whether they are in an excited state or not? We have proved in model reactions earlier [4, 5] that these aldehydes are formed in excited states in the presence of L-lysine and hydrogen peroxide. Besides the excited aldehydes singlet oxygen formation and chemiluminescence could be observed, too.

The liberated excited aldehydes (activated aldehydes) are extremely reactive radical compounds which can attack the cellular components like proteins, RNA, DNA, etc. The formed singlet oxygen is very rich in energy having 147.35 kJ/mol more energy than normal oxygen, thus it can oxidize free amino acids, proteins, nucleic acids, especially guanine, guanosine, forming 8-hydroxy-guanosine [6, 7].

The model reactions were adapted and extended to the S9 fractions of rat liver [2]. In this way, it was possible to investigate the liberation of excited aldehydes from carcinogenic compounds.

Materials and Methods

The spectral distribution of the chemiluminescence (CL) was analyzed by the spectrometer Model VG-05 developed at the Central Research Institute for Chemistry of the Hungarian Academy of Sciences in Budapest, in a mixture including

L-lysine: CH_2O: H_2O_2(formaldehyde)	(1mM: 1mM: 1mM)
L-lysine: CH_3-CHO: H_2O_2(acetaldehyde)	(1mM: 1mM: 1mM)
L-lysine: OHC-CHO: H_2O_2(glyoxal)	(1mM: 1mM: 1mM)
L-lysine: CH_3-C(=O)-CHO: H_2O_2(methyl-glyoxal)	(1mM: 1mM: 1mM)
L-lysine: CH_2=CH-CHO: H_2O_2(acrolein)	(1mM: 1mM: 1mM)
L-tryptophane: CH_2O: H_2O_2	(1mM: 1mM: 1mM)
L-tryptophane: OHC-CHO: H_2O_2	(1mM: 1mM: 1mM)

at $T = 298$ K, pH = 7.4 in 0.1 M Sörensen buffer.

Transmitting chemiluminescence radiation through a series of coloured glass filters having different cut-off wavelength characteristics enabled the spectral profile of the emission to be calculated, thus pinpointing the emitter even in complex situations (see *Fig. 1*).

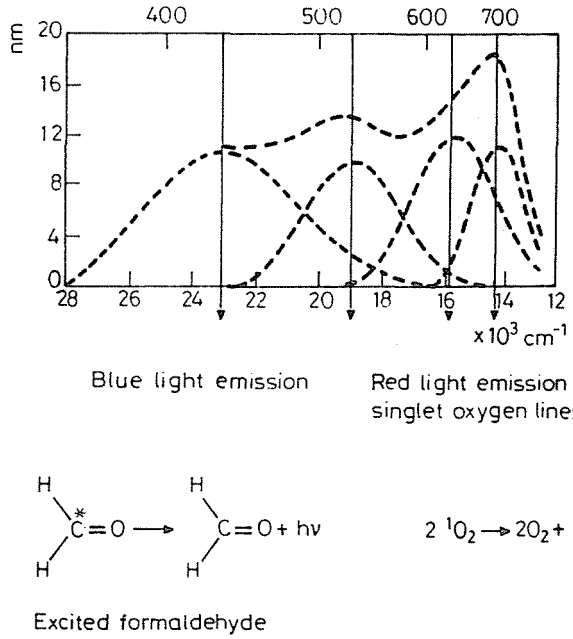


Fig. 1. Light emission analysis at different wavelengths in a mixture of L-lysine: CH_2O: H_2O_2 (1mM: 1mM: 1mM) at T=298 K, pH=7.4 in 0.1M Sörensen buffer.

Post-mitochondrial S9 Fractions

The metabolic activation system [2] used most widely in short-term bioassays is the 9000×g supernatant fraction (S9) of liver from Wistar: Han: Lati male rats, weighing 200–250 g, pretreated with polychlorinated biphenyls (Aroclor 1254, Analabs J 147A) 1×500 mg/kg, i.p. induction.

S9 Mix (0°)

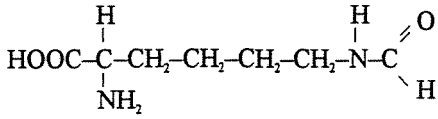
10 % S9 fraction (protein content 29.0 mg/ml) according to LOWRY [8]; 50 % Na-phosphate buffer (0.2 M, pH=7.4); 40 % NADPH generating system (cofactor solution): 33 mM KCl, 8 mM $MgCl_2$, 5 mM glucose-6-phosphate, 4 mM NADP. S9 mix contains oxygenase enzymes which are important to metabolize the xenobiotics.

Xenobiotics applied in S9 mix: DMN (dimethyl-N-nitrosamine), MMH (monomethyl N-hydrazine), HZ/FORM (hydrazine-formaldehyde adduct), INH/FORM (isonicotinic acid-hydrazide-formaldehyde adduct). The final concentration of xenobiotics in S9 mix was 1 mM.

Results

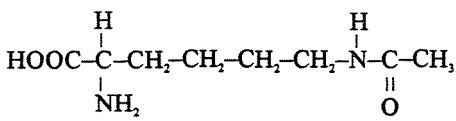
The measured CL-spectrum can be seen in *Fig. 1*. Singlet oxygen lines and a band at 23.000 cm^{-1} (430 nm) characterizing the $^3A_2 \rightarrow {}^1A_1$ transition of excited formaldehyde can be identified.

Analyzing the reaction mixture with MS and NMR a considerable amount of N^{ϵ}-formyl-L-lysine besides N^{ϵ}-methyl-L-lysine can be detected.



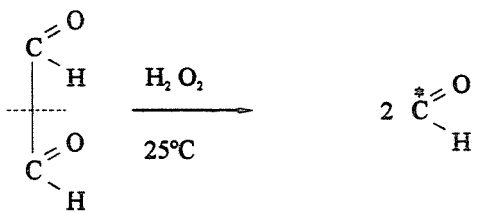
N^{ϵ}–formyl–L–lysine

We have also investigated acetaldehyde as well as glyoxal using similar methods and reaction parameters. Acetaldehyde gave similar results to formaldehyde and N^{ϵ}-acetyl-L-lysine was detected in the reaction mixture by MS.

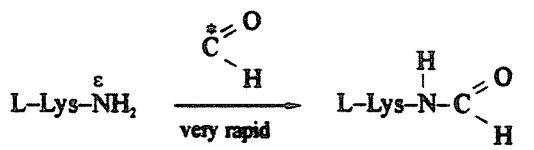


N^{ϵ}–acetyl–L–lysine

Glyoxal gave ten times higher chemiluminescence and N^{ϵ}-formyl-L-lysine has been detected in the reaction mixture, showing that H_2O_2 can disrupt glyoxal by forming formyl radicals.



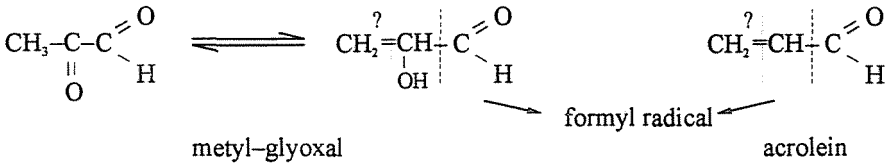
glyoxal formyl–radical



very rapid

N^{ϵ} formyl–L–lysine

In the case of methyl glyoxal and acrolein the same compound N^{ϵ}-formyl-L-lysine has been found in the reaction mixture by radiochemical method.



It was found that peptides containing L-lysine, caseine (containing 10 mol% lysine) and cytochrome C from horse heart also show an increased chemiluminescence. It is noteworthy that L-tryptophane gives a similar effect to L-lysine, but no effect was observed with L-cysteine and L-arginine.

The reaction mixtures of tryptophane with formaldehyde and H_2O_2 as well as glyoxal and H_2O_2 gave a burst chemiluminescence and formed the very same product N-formyl-tryptophane. This compound had been identified first in a biological model reaction.

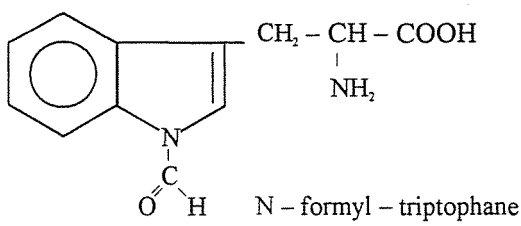


Fig. 2 shows clearly that those carcinogenic compounds which generate formaldehyde or contain bonded formaldehyde (e.g. HZ/FORM) show a burst chemiluminescence (originating from excited aldehydes and singlet oxygen) in S9 liver fractions.

From dimethyl-nitrosamine (DMN) and mono-methyl-hydrazine (MMH) excited formaldehyde is liberated only after 30 min preincubation, whereas from hydrazine-formaldehyde adduct (HZ/FORM) and isonicotinic acid-hydrazine-formaldehyde adduct (INH/FORM) it is liberated immediately; no preincubation time is necessary in these cases.

These results may correlate with the methylating capacity of the above carcinogenic compounds. The liberated excited formaldehyde can play a role in the methylation processes [3], since JENSEN et al. [9] have found a correlation between the *in vitro* methylation of DNA by microsomally activated dimethyl-nitrosoamine and the liberated formaldehyde.

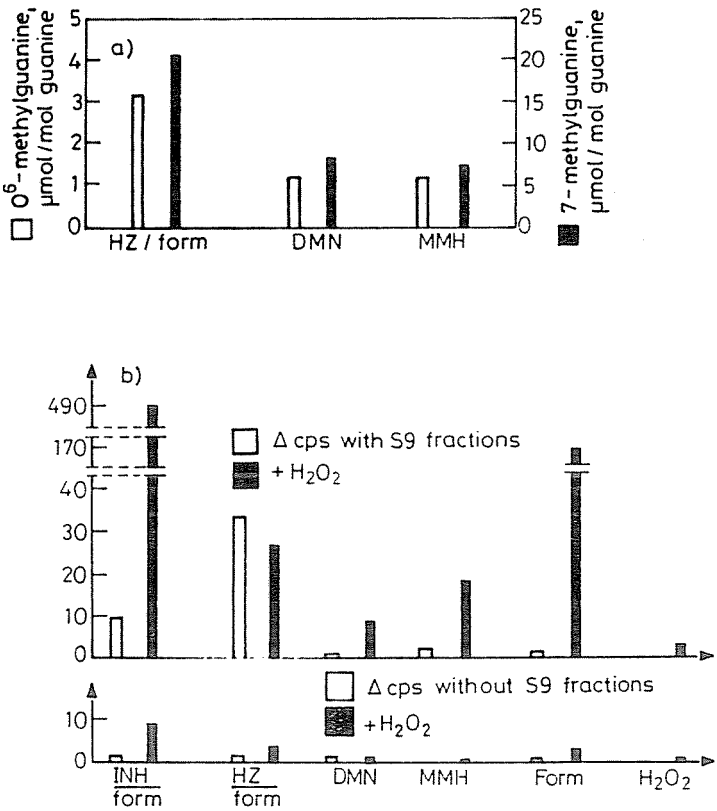
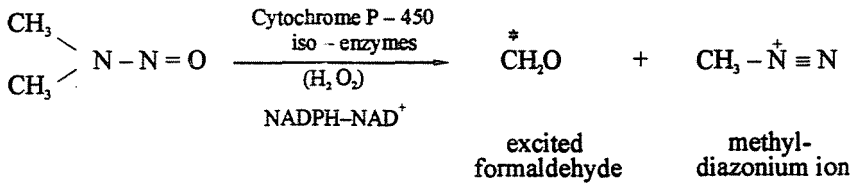


Fig. 2. Metabolic activation of carcinogenic compounds by S9 rat liver cell fractions.
2.a. *In vitro* methylation of calf thymus DNA with different carcinogenic compounds by rat liver cell fractions according to Ref. [14].
2.b. Chemiluminescence emission of excited formaldehyde + singlet oxygen from different carcinogenic compounds by *in vitro* metabolic activation of S9 rat liver cell fractions.



N, N – dimethyl – nitrosamine

Other literature data [10–14] support also this conception (see *Fig. 2*), as these carcinogenic compounds strongly methylate DNA, forming O^6-methyl and N^7-methyl-guanines and at the same time these compounds are formaldehyde precursors in rat liver. These methylated nucleic bases can be formed only in cancerous tissues. Very interesting results can be found in Ref. [13], which are to be taken into consideration: the ability of enzymatic removal of O^6-methyl-guanine from DNA in rat liver showed a striking change in response to the dose of the administered carcinogenic DMN, by injuring the removal enzymatic system.

These results coincide with our investigations, as the liberated excited formaldehyde and singlet oxygen may attack the repair enzyme system.

Three famous biochemical institutes (Karolinska Institute, Stockholm; National Cancer Institute Bethesda, Maryland; Institute of Biochemistry, University of Graz, Austria) have worked together on these problems and proved that formaldehyde, acetaldehyde and acrolein inhibit the activity of O^6-methyl-guanine DNA methyl-transferase enzyme, that is they inhibit the repair enzyme, thus the methyl group of O^6-methyl guanine cannot be removed.

The most reactive aldehyde is acrolein, which is accumulated in polluted air besides formaldehyde and acetaldehyde [15–20].

Important results are shown in the second part of *Fig. 2*. The hydrazine-formaldehyde adduct exhibits a huge chemiluminescence effect. This compound can methylate DNA and it can be considered as a strongly carcinogenic compound [14].

It is especially interesting that not only hydrazine and formaldehyde adduct cause a large chemiluminescence effect, but isonicotinic acid-hydrazide (INH), the well-known ISONIAZID antituberculoticum, shows a similar burst chemiluminescence. It has been published in *Nature* already in 1962 [21] that ISONIAZID caused pulmonary tumors in mice, similarly to hydrazine.)

It would be extremely important to reinvestigate the biological effect of hydrazine-group containing medicines, e.g. Depressan (1,4-dihydrazino-

phthalazine), PAS-hydrazide, Veratryl-hydrazine, Lisidomil, etc., since these drugs may be health hazards and may have side effects.

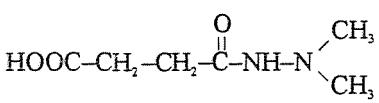
The hydrazine molecule is very reactive towards formaldehyde, it can react with the *'endogenous formaldehyde'* in C_1-pool and can eliminate the indispensable formaldehyde from the human body, forming endogenous carcinogenic formaldehyde adduct instead. It was shown in 1988 [14] that hydrazine alone can methylate DNA, forming O^6-methyl- and N^7-methyl-guanines. The origin of methyl groups can be assigned only to the endogenous formaldehyde.

Discussion

The results obtained prove that excited formaldehyde and singlet oxygen can be liberated from various carcinogenic compounds in rat liver cell fractions, similarly to the model reactions. The model reactions studied by us in lysine containing solutions are also relevant to biological circumstances due to the extremely high lysine concentration (12–15 %) in liver.

These compounds can get *exogenously* into human organisms from the environment with food, fruit, drinking water, air, tobacco smoke, pesticides and medicines. They can also be formed *endogenously* in the human body from compounds containing hydrazine group with endogenous formaldehyde in C_1-pool. Due to the above facts it is necessary to lower the risk of contact of these hazardous compounds with the human population.

A similar hazardous compound (ALAR, B9) was in practical use in the U.S.A. in agriculture as a growth regulator for many years. ALAR is a hydrazine derivative



which has been widely used on apples since the late 1960s to promote a uniform red color and prolong shelf life. It keeps the apples longer on tree, but it penetrates into the apple skin and cannot be washed off. Since 1985 ALAR's use has dropped substantially [22, 23]. It is probable that ALAR may have caused a cancer risk first of all at children [22, 23], as children eat relatively more fruit compared to their body weights than adults.

In the highlight of our results it can be concluded that in the future chemicals like pesticides, medicines, etc. have to be controlled in the liver detoxication mechanism in respect of liberating dangerous excited formaldehyde (aldehydes), singlet oxygen, etc.

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Erratum

In Vol. 36, No. 3 the paper V. Horváth, L. Trézl, T. Szarvas, J. Pipek, Cs. Vida, K. Bauer: 'Investigation of Cyano-Methylation Reaction by Cyanohydrine and its Determination in Tobacco-Smoke (Strecker-reactions)' appeared with an incomplete reference list. The missing items are as follows:

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