AUTOXIDATION OF ALKYLHYDRAZONES AND MUTAGENICITY OF THE RESULTING HYDROPEROXIDES

Masataka MOCHIZUKI,* Kozo MICHIGAMI, Katsuko SHIOMI, Kei ITOH, Yoshiko TANGE and Shoji HIZATATE
Kyoritsu College of Pharmacy, Shibakoen 1-5-30, Minato-ku, Tokyo 105, Japan

Acetone alkylhydrazones were readily autoxidized to 2-alkylazo-2-propyl hydroperoxides, which were directly mutagenic in Salmonella typhimurium TA1535, TA100, TA102 and Escherichia coli WP2hcr. The mechanism of this mutagenicity presumes that the hydroperoxides in aqueous solution decompose to alkyl diazonium ions which were observed in the alkylation of 4-(p-nitrobenzyl)pyridine, and also to hydroxyl radical which was detected by ESR.

KEYWORDS alkylhydrazone; mutagenicity; autooxidation; hydroperoxide; alkylation; hydroxyl radical

Hydrazines and their derivatives are environmental carcinogens; alkylhydrazones are also found in our environment, and some of them are carcinogenic.1) We found that alkylhydrazones were mutagenic in the presence of air, and the present paper describes the autoxidation of acetone alkylhydrazones and the mutagenicity of their products in Salmonella typhimurium and Escherichia coli in relation to the metabolic activation of alkylhydrazines.

The alkylhydrazones have no direct mutagenicity by themselves; however, acetone methylhydrazone was mutagenic only in the presence of oxygen, and its mutagenicity in S. typhimurium TA100 and TA102 increased by bubbling of oxygen. The conversion of acetone methylhydrazone by bubbling of oxygen was observed in 1H-NMR spectrum. Decreases in three singlets and one broad signal of acetone methylhydrazone and increases in two sharp singlets and one broad signal of the new product were observed.2) This new compound, produced from acetone methylhydrazone and oxygen, was estimated to be 2-methylazo-2-propyl hydroperoxide. This structure was supported by 1H-NMR and IR spectra, and by the chemical behavior of liberating iodine from iodide. We found the hydroperoxide to be explosive and avoided thorough purification.

© 1993 Pharmaceutical Society of Japan
From the $^1$H-NMR spectrum, the hydroperoxide contained acetone and methanol as impurities$^3$ in quantities of 2 to 8%. Similar structures were reported by Pausacker in the autooxidation of phenylhydrazones.$^4$ Among four alkylhydrazones$^5$ prepared from acetone or acetaldehyde and methylhydrazine or dimethylhydrazine, only acetone methylhydrazones produced the azohydroperoxide. No change in $^1$H-NMR spectrum was observed in the other three hydrazones, suggesting that ketone monoalkyl hydrazine structure is required for the autooxidation. 2-Methylazo-2-propyl hydroperoxide was mutagenic in $S.$ typhimurium TA1535, TA100, TA102, and E. coli WP2/hr ($^($Fig. 1$^)$).

The mechanism of mutagenicity of the hydroperoxide was investigated. Since cumene hydroperoxide and tert-butyl hydroperoxide were weakly mutagenic only in $S.$ typhimurium TA102, but not mutagenic in strains TA1535 and TA100, mutagenicity of 2-methylazo-2-propyl hydroperoxide did not derive from hydroperoxide structure alone. Three more 2-alkylazo-2-propyl hydroperoxides were prepared from ethyl-, propyl- and butylhydrazine in the reaction with acetone,$^9$ and the effect of alkyl group on mutagenicity was examined in $S.$ typhimurium TA1535 and E. coli WP2/hr. The hydroperoxide having methyl group was the strongest mutagen in $S.$ typhimurium TA1535. Other hydroperoxides having ethyl, propyl and butyl group were strongly mutagenic in E. coli WP2/hr ($^($Fig. 2$^)$). The effect of alkyl group on mutagenicity in two bacterial strains was similar to that in $N$-nitroso-$N$-(hydroxymethyl)-alkylamines$^7$ which are metabolically activated structures of $N$-nitrosodialkylamines, and also to that in alkane diazohydroxides$^8$ which are ultimate alkylating species of many alkylating carcinogens. Alkylation abilities of four

![Graph 1](image1)

![Graph 2](image2)

![Graph 3](image3)

![Chart](image4)

**Fig. 2.** Mutagenicity of 2-Alkylazo-2-propyl Hydroperoxides (alkyl = methyl (○), ethyl (●), propyl (▲) and butyl (■)) in Salmonella typhimurium TA1535 (A) and E. coli WP2/hr (B)

**Fig. 3.** Alkylation Activity of 2-Alkylazo-2-propyl Hydroperoxides Expressed by the Visible Absorbance of Alkylated NBP (alkyl = methyl (○), ethyl (●), propyl (▲) and butyl (■))

**Chart 1.** Possible Pathway of Mutagenic Activation of Acetone Alkylhydrazone
2-alkylazo-2-propyl hydroperoxides determined by a 4-(p-nitrobenzyl)pyridine (NBP) assay\(^9\) varied among the alkyl derivatives, and also depended on their concentrations or reaction periods. Among four hydroperoxides, 2-methylazo-2-propyl hydroperoxide had the highest alkylating activity (Fig. 3).

On the other hand, formation of radical species from hydrazines was reported.\(^{10}\) We detected the formation of hydroxyl radical spontaneously from 2-alkylazo-2-propyl hydroperoxides by ESR.\(^{11}\)

In conclusion, acetone monoalkyhydrazones are autoxidized to produce 2-alkylazo-2-propyl hydroperoxides. The hydroperoxides decompose to acetone and presumed alkyl diazonium ions.\(^3\) The alkyl diazonium ions can alkylate DNA and show mutagenicity in bacteria. Another decomposition produces hydroxyl radical, which can damage DNA\(^{12}\) (Chart 1).

ACKNOWLEDGEMENT We are grateful to JEOL Ltd. for spin trapping experiments by ESR. A part of the present work was supported by a Grant-in-Aid from the Science Research Promotion Fund of Japan Private School Promotion Foundation.

REFERENCES AND NOTES


2) Three singlets (δ 1.7, 1.8 and 2.9 ppm) and one broad signal (δ 4.3 ppm) of acetone methylhydrazone were decreasing and two sharp singlets (δ 1.5 and 3.8 ppm) and one broad signal (δ 9.2 ppm) were increasing.

3) 2-Alkylazo-2-propyl hydroperoxides decomposed in D$_2$O to alcohols and acetone which was detected in $^1$H-NMR.


6) 2-Alkylazo-2-propyl hydroperoxides were prepared by bubbling of oxygen into dichloromethane solution of acetone alkylhydrazones for 2h at room temperature. $^1$H-NMR(CDCl$_3$) of 2-alkylazo-2-propyl hydroperoxides, methyl: δ 1.46(s, 6H), 3.82(s, 3H), 9.19(s, 1H), ethyl: δ 1.32(t, J=7.3Hz, 3H), 1.44(s, 6H), 3.89(q, J=7.3Hz, 2H), 9.45(s, 1H), propyl: δ 0.96(t, J=7.3Hz, 3H), 1.45(s, 6H), 1.85(m, 2H), 3.82(q, J=7.3Hz, 2H), 9.45(s, 1H), butyl: δ 0.95(t, J=7.3Hz, 3H), 1.43(m, 2H), 1.45(s, 6H), 1.79(m, 2H), 4.05(q, J=7.3Hz, 2H), 9.41(s, 1H).


11) ESR spectrum were recorded in a flat cell using JEOL JES-RE3X spectrometer. Reactions were initiated by mixing of 150μl of 0.1M phosphate buffer (pH 7.4), 5μl of 2-alkylazo-2-propyl hydroperoxide (0.4 to 40μmol) and 50μl of 5,5-dimethyl-1-pyrroline N-oxide(DMPO) at room temperature, and spectra were taken 1min after the mixing. DMPO-OH adducts disappeared when methanol was added as hydroxyl radical scavenger. Spin concentration of DMPO-OH adducts depended on the concentration of 2-alkylazo-2-propyl hydroperoxide.