SPIN TRAPPING IN BIOLOGICAL SYSTEMS. OXIDATION OF THE SPIN TRAP 5,5-DIMETHYL-1-PYRROLINE-1-OXIDE BY A HYDROPEROXIDE-HEMATIN SYSTEM*

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SUMMARY

We have used the spin trap 5,5-dimethyl-1-pyrroline-1-oxide to determine if primary free radicals are involved in the hematin-cumene hydroperoxide system which has been shown to oxidize N-hydroxy-2-acetylaminofluorene into the nitroxyl free radical form of this carcinogen. We have found that the spin trap was oxidized itself rather than trapping either primary free radicals or carcinogen free radicals.

INTRODUCTION

Spin trapping is a new technique which has potential in helping to unravel the molecular mechanisms involved in reactions having rapidly reacting free radical intermediates. Janzen (1) and Lagercrantz (2) have summarized many of the earlier observations involving this technique. Recently Bolton and coworkers (3,4) have applied this technique to biological systems. In short the technique involves the ability of the spin trap to react with free radicals which have a short lifetime to yield a spin adduct having a longer lifetime. The ESR spectrum of the spin adduct yields information hopefully specifically identifying the original free radical. Aryl nitrones and nitroso compounds have been used as spin traps (1,2).

We have discovered that the carcinogen N-hydroxy-2-acetylaminofluorene (N-OH-AAF) is oxidatively activated via an obligatory nitroxyl free radical form of the carcinogen in either a linoleic acid hydroperoxide-hematin (5) or a cumene-hydroperoxide-hematin system (6). Since these observations may be of importance to the understanding of the activation of a large class of carcinogens (aryl-amine class), we thought that a primary free radical(s) produced in the hydro-

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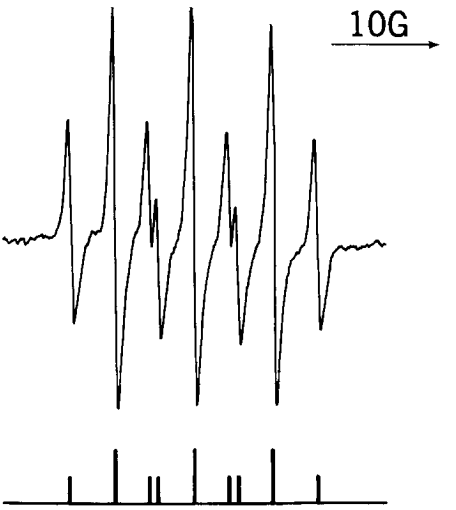


Figure 1:

The electron spin resonance spectrum obtained when 240 μM of cumene hydroperoxide was added to a pH 7.4 0.015 M potassium phosphate buffer containing 20 μM hematin and 40 μM DMPO. The microwave power was 20 m Watts and the modulation frequency was 100 k Hz with an amplitude of 0.5 guass. The sweep time was 1.67 gauss per minute and the response time was 10 sec. The g value of the radical is 2.0065 with A_N = 7.1 gauss and A_H = 4.2 gauss.

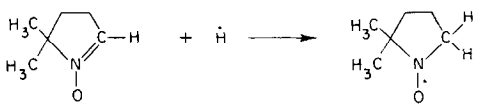
peroxide-hematin reaction such as perhaps OH radicals may be acting as the primary oxidant and that other cellular reactions producing the same free radicals may also serve to activate these carcinogens. Spin trapping agents seemed a likely technique to test these ideas. We report our results here. They are of general importance because: A) they provide a caveat in that this is an example of a spin trap acting in a reaction in a manner not a priori considered, B) reinforce our previous ideas concerning N-OH-AAF oxidative activation mechanisms and C) illustrate that the spin trap does not trap the carcinogen nitroxyl free radical intermediate.

METHODS

The carcinogens were synthesized according to the methods we have described previously (7,8). Hematin from ox blood was purchased from Sigma Chemical Company and cumene hydroperoxide from Matheson Coleman and Bell. The methods involved in starting the reactions and of obtaining ESR spectra were as previously reported (5). DMPO (5,5-Dimethyl-1-pyrroline-1-oxide) was synthesized according to methods in the literature (9).

RESULTS AND DISCUSSION

Figure 1 presents the ESR spectrum obtained when, to a solution containing DMPO and hematin, cumene hydroperoxide was added. The free radical obtained has a g value of 2.0065. It is possible to explain the spectrum obtained by assuming that the free electron is on the nitroxide bond and that its orbital interacts with two equivalent protons. The hyperfine coupling constants are a_N = 7.1 gauss and a_H = 4.2 gauss. The observed hyperfine coupling constants do not agree with that reported for the hydrogen atom aduct to DMPO (10), (a_N = 14.43 gauss, a_H^B = 18.89 guass), ie.



which would have a spectrum indicative of the free electron interacting with two equivalent protons. Our results can be explained by assuming that hematin and cumene hydroperoxide oxidizes DMPO into the oxidation products as such:

The oxygen effect will be explained later. The oxidized product which we will refer to as DMPOX, is 5,5-dimethyl-pyrrolidone-(2)-oxyl-(1) following the nomenclature of Aurich and Trosken (11) who synthesized the compound and compared the hyperfine coupling constants in different solvents. They did not obtain the values in water, but the values obtained in methanol are approaching those we obtained in water and our values are also consistent with the expected values using their developed relationship of hyperfine coupling constants as a function of solvent polarity. It should be noted that DMPOX would yield hyperfine splitting constants from the two equivalent protons on the number 2 carbon and as such is consistent with the two equivalent protons suggested by the observed spectra.

Figure 2 presents the results of experiments which indicate that an oxidizable carcinogen competes with DMPO as a substrate in the hematin-peroxide

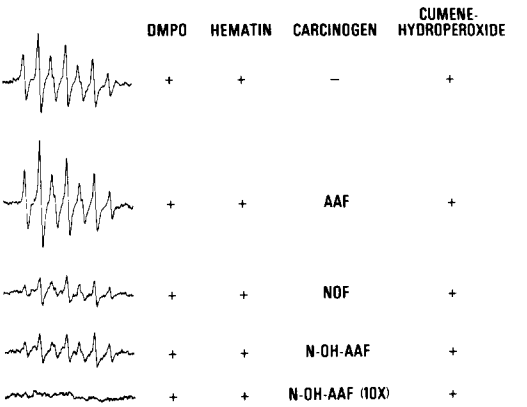


Figure 2:

The electron spin resonance spectra obtained on the DMPO hematin cumene hydroperoxide system as described in Figure 1 after the addition of 40 μM of various arylamine carcinogens. AAF refers to 2-acetylaminofluorene, NOF to 2-nitrosofluorene and N-OH-AAF to N-hydroxy-2-acetylaminofluorene. In the lowest trace 400 μM of N-OH-AAF was added and in the upper trace no carcinogen was present.

system. DMPO added to the hematin-peroxide system yielded the spectrum shown in the upper trace which is due to DMPOX. If the carcinogen 2-acetylamino-fluorene (AAF) was present then the second trace was obtained. AAF is not oxidized by this system and indeed the amount of DMPOX formed is as much as if not more than that formed in the absence of AAF. If the more active carcinogens 2-nitrosofluorene (NOF) or N-hydroxy-2-acetylaminofluorene (N-OH-AAF) are added to the system then the spectra obtained indicated less DMPOX was formed. These two carcinogens are oxidized in this system (6). If much more (10 times more) N-OH-AAF was added then this completely prevented DMPOX formation (lowest trace).

Figure 3 demonstrates that the presence of oxygen was necessary to form DMPOX in the hematin-peroxide system. We do not know the exact molecular events that eventually yield DMPOX, but these results do indicate that oxygen addition to DMPO may come from molecular oxygen. There are of course other possibilities such as A) the oxygen may come from cumene hydroperoxide, or B) from water. These possibilities are now being tested.

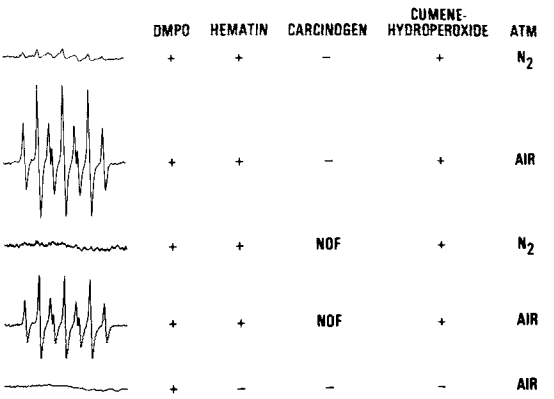


Figure 3:

The electron spin resonance spectra obtained on the DMPO hematin cumene hydroperoxide system of Figure 1 as affected by an air or nitrogen atmosphere and by the presence or absence of 40 μM of 2-nitrosofluorene.

The present report does demonstrate that in a system where we a priori postulated, the existence of primary free radicals, the spin trap DMPO did not trap free radicals but was oxidized itself. Also it is known that the nitroxyl free radical of N-OH-AAF is produced in the hematin-peroxide system, but we do not have evidence to indicate that the spin trap DMPO is trapping this carcinogen free radical. The observation that the oxidizable carcinogens NOF and N-OH-AAF did decrease the formation of DMPOX and that the non-oxidizable carcinogen AAF did not decrease DMPOX formation indicates that DMPO is competing with oxidizable substrates for an active oxidant species in this system. The fact that we have not yet been able to trap a primary radical such as OH radical doesn't mean that these species are absent, it merely means that the probability of DMPO being oxidized is much higher than it trapping a primary radical and this can be explained by the differences in the rates of formation and decay of trapped radicals versus the rate of oxidation of DMPO and the stability of its oxidation product, DMPOX.

It is anticipated that spin-trapping will add substantially to our knowledge of reactions involving free radicals; the results presented here may be

useful to help explain anomolous results or helpful in the eventual design of better spin traps.

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