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Generation of Free Radicals from Dihydropyrazines with DNA Strand-Breakage Activity

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Abstract: ESR spin-trapping techniques revealed that free radical species were generated in a buffer solution (pH7.1) of compounds (**1** ~ **5**) having a dihydropyrazine skeleton. Oxygen radicals and several carbon-centered radicals were detected as adducts of spin traps: DMPO and DBNBS. Secondary and tertiary radicals trapped were assigned to the carbon-centered radical structures.

In previous papers[1,2], we showed that 2,5-bis (D-arabino-tetrahydroxybutyl) dihydropyrazine was formed in an aqueous solution of D-glucosamine and that some dihydropyrazine derivatives, including this compound and others, which were synthesized independently, like 2,3-dihydro-5,6-dimethylpyrazine (**1**), had much greater DNA strand-breaking activity in plasmid pBR322 than D-glucosamine itself. Later, it was found that certain carbon-centered radicals were generated in an aqueous solution of D-glucosamine[3].

In the present work, therefore, we have tried to detect the generation of oxygen radicals and certain carbon-centered radicals in an aqueous solution of some dihydropyrazine derivatives using an ESR spin-trapping technique with 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and sodium 3,5-dibromo-4-nitrosobenzensulfonate (DBNBS). Compound (**1**), 2,3-dihydro-2,5,6 -trimethylpyrazine (**2**), 2,3-dihydro-2,2,5,6-teramethylpyrazine (**3**), trans-2,3-dimethyl-5,6,7,8,9,10-hexahydroquinoxaline (**4**) and cis-2,3-dimethyl-5,6,7,8,9,10-hexahydroquinoxaline (**5**) were synthesized by the method[2] described earlier. As the Tris-HCl buffer has no effect on the generation of radicals, the ESR spectra were measured in a 50mM Tris-HCl buffer (pH7.1) in the presence or absence of the cupric ion (Cu^{2+}) used in the reaction conditions of DNA strand breakage. All the compounds (**1**, **2**, **3**, **4** and **5**) revealed approximately the same signal pattern of the DMPO-adduct, with various levels of intensity. The ESR spectrum observed consists of four DMPO-adducts: the hydroxyl radical[4,5] (the quartet lines), two carbon-centered radical adducts (the doublets of sixtet lines) which were characterized by the parameters g = 2.0060, hfsc: aN = 1.61mT, aH = 2.31mT and g = 2.0056, aN = 1.53mT, aH = 1.88mT, respectively, and the residual four signals might be due to the hydroperoxyl radical ($\cdot$ OOH),[3,4,5] in which only the edges of the signals appeared. The presence of these carbon-centered radicals was confirmed by their DBNBS-adducts. Figure 1 shows the ESR spectrum of the DBNBS-adduct of **2**; the signal patterns in both cases (with/without Cu^{2+}), were in accordance with each other. The ESR spectrum observed consists of two DBNBS adducts marked ε and μ for their carbon-centered radicals (ε: $\cdot CHR_2$: g =

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2.0063, aN = 1.38 mT, aHβ = 2.00 mT, aHmeta = 0.0 7 mT and μ: • CR_3: g = 2.0063, aN = 1.38 mT, aHmeta = 0.07 mT). Since both g values were the same, it was expected that all the signals were due to a sole first carbon radical species: • CH_2R. However, three signals marked μ could be separated from the sixtet

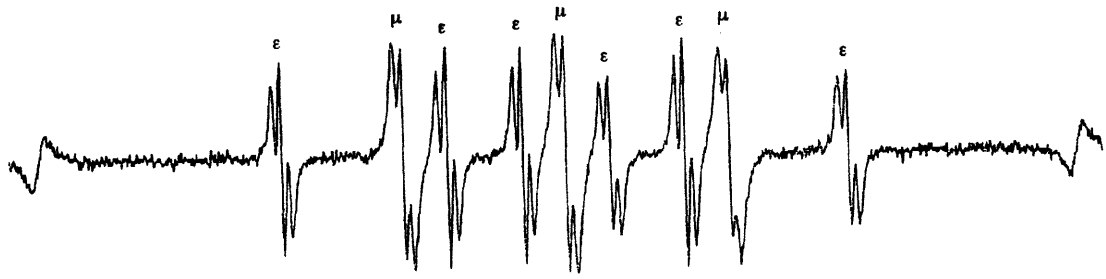

Fig. 1. The ESR Spectrum of DBNBS-Adduct of 2,3-Dihydro-2,5,6-Trimethypyrazine(2)
The measured solution consist of 2μl cupric chloride aqueous solution (1mM), 10μl aqueous solution of DBNBS (20mg/200μl), 20μl aqueous solution of 2,3-dihydro-2,5,6-trimethylpyrazine (2) (3μl/ 500μl) and 168μl 50mM Tris-HCl buffer (pH7.1). The ESR spectrum was recorded at 10min after mixing at room temperature; microwave power = 8mW, microwave frequency = 9.41GHz, sweep time = 8min, receiver gain = 5x100, field modulation width =1x0.01 and time constant = 0.1. The signals (g2 = 2.033 and g1 = 1.981) of Mn2+ marker were recorded at the edges on both sides in the spectrum. The signals marked with ε and μ were the adducts of • CHR2 and • CR3, respectively.

signals marked ε because the three μ signals showed a stronger peak height than the ε signals, immediately after the start of the record, furthermore, two DMPO-adducts, due to carbon-centered radicals, were detected. Thus, the three μ signals were assigned to a tertiary carbon-centered radical adduct and the six ε signals to a secondary carbon-centered one. In contrast to the spectra of the DMPO-adduct, the DBNBS-adduct spectra of dihydropyrazines listed above showed alternative signal patterns which were due only to certain carbon-centered radicals but not to the agreement between **1** and **2**, and no oxygen radicals were observed. All the compounds listed showed the data assigned on the DBNBS-adducts which were secondary and tertiary carbon-centered radicals. All the compounds generated plural radicals which showed the g value 2.0062 to 2.0068.

From our results, it became apparent that some dihydropyrazines (**1**~**5**) generated certain carbon-centered radicals in an aqueous solution under the condition where they exhibited DNA strand-breakage activity. Therefore, the carbon-centered radicals were considered to be the reactive species in the DNA strand breakage. It can also be predicted that those which were secondary or tertiary carbon radicals will affect the specificity of DNA strand breakage by various bulky states of the radical. Studies on the preferred breakage site in the DNA by these dihydropyrazines are in progress.

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