RADIATION-INDUCED DNA BASE DAMAGE: MECHANISMS OF FORMATION OF 8-HYDROXYDEOXYGUANOSINE FROM 2'-DEOXYGUANOSINE BY HYDROXYL RADICALS

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Ionizing radiation produces highly reactive hydroxyl (OH) radicals which induce oxidative stress in living cells. When DNA is exposed to ionizing radiation (X- or γ-rays), the change of 2'-deoxyguanosine (2'-dG) to 8-hydroxydeoxyguanosine (8-OHdG) is induced and the resulting 8-OHdG is regarded as a base alteration. Since recent studies indicate that the formation of 8-OHdG in DNA by OH radicals is closely related to the radiation-induced oxidative stress in the cells, it is important to elucidate the mechanisms of the change of 2'-dG to 8-OHdG induced by OH radicals.

Chemically synthesized 8-OHdG was used as a standard. A method combining HPLC and an electrochemical detector (ECD) was found to be quite useful for detecting and identifying OH-radical-induced 8-OHdG.

OH radicals were prepared by irradiating an N_2O-saturated aqueous solution with γ-rays. When an N_2O-saturated aqueous solution containing 8 mM of 2'-dG was exposed to γ-rays and analyzed by HPLC with ECD, 8-OHdG was found and the yield of 8-OHdG increased with the increase in the concentration of OH radicals. To obtain information concerning the mechanisms of formation of 8-OHdG from 2'-dG, an aqueous solution containing 8 mM 2'-dG was exposed to γ-rays in the presence or absence of 8 mM α-phenyl-*t*-butylnitrone (PBN). In each case, the concentrations of OH radicals that actually reacted with 2'-dG were calculated by using reaction-rate constants of 2'-dG and PBN with water radicals (OH radicals and hydrated electrons), which were determined by the nanosecond pulse-radiolysis method. From the relationship between the concentration of OH radicals and the amount of 8-OHdG, the yield of 8-OHdG, in the presence of PBN was found to be twice that in the absence of PBN. An examination of the solution containing 2'-dG and PBN or PBN alone after γ irradiation by ESR spectroscopy provided evidence that one OH radical was added to the N7-C8 double bond of the guanine base to form 8-OHdG when PBN was present in the solution, whereas in the absence of PBN two OH radicals reacted with the double bond of the base to form 8-OHdG.