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8-Hydroxyguanine, A Product From Damaged DNA Can Be Used in the DNA Salvage Pathways

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Abstract: The metabolic mechanism of 8-hydroxyguanine (8-OHGua) is so far unknown, however it is known to function in the mutagenic events in biological systems. In the present study, the metabolic processes is investigated by the reaction of 8-OHGua with PRPP catalyzed by HGPRT and Mg(II). The evidence shows that the interaction between 8-OHGua and the enzyme is indeed taken place and 8-OHGMP is formed.

Keywords: 8-Hydroxyguanine, metabolic reaction, DNA salvage pathway.

8-Hydroxyguanine (8-OH-Gua) is one of many lesions generated in DNA damaging by oxidative processes including free radicals. It is the most extensively investigated lesion, due to its miscoding properties and its potential role in mutagenesis, carcinogenesis and aging[1,2] . It has been reported that the accumulation of 8-OHGua will induce G:C to T:A transversion during DNA replication[1] that is related to mutagenic events[3,4] . Although some repair enzymes such as glycosylase/endonuclease activity in *Escherichia coli* can remove 8-OHGua from DNA[5], most of 8-OHGua induced by reactive oxygen species (ROS) have its own metabolic pathway to be used as producing mutation in *vivo*. The metabolic mechanism of 8-OHGua is unknown. Therefore, the pathway how the accumulated 8-OHGua by DNA damage become metabolized stand a foundational and predominant position. The present report is the first of such investigation for the reaction of 8-OHGua with PRPP catalyzed by HGPRT and Mg(II). The results presented indicate that 8-OHGua can be used by DNA synthesis. That implicates that 8-OHGua, probably, can produce the mis-incorporation of DNA directly by DNA by salvage pathway.

Experimental

Materials

8-Hydroxyguanine (8-OHGua), hypoxanthine-guanine phosphoribosyltransferase (HG

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PRTase), α-D-5-phosphorbosyl-pyrophosphate (PRPP) were purchased from the Sigma Chemical Co (USA). Other agents are A. R grade.

Reaction of HGPRTase with 8-OHGua

To test the possibility that 8-OHGua is salvaged, the interaction between 8-OHGua and the enzyme and formation of 8-OHGMP from 8-OHGua were studied. 8-OHGua (1.5 μmol/L) was incubated with HGPRTase (19.2 μg) at 30°C in 3.0 mL that contain 5 mmol/L PRPP, 12 mmol/L $MgCl_2$, 100 mmol/L Tris-HCl at pH7.4. After 30 min incubation, an aliquot (100 μL) was used for circular dichroism (JASCO, J-715 spectropolarimeter, Japan) and the remaining solution was filtered through Milllipore (Biomax: 10K). A part of the filtrate (100 μL) was used to observed the spectrum change of the reaction of 8-OHGua and PRPP (500 μL) and the remaining filtrate was used for mass spectroscopic study to detect the formation of 8-OHGMP. The filtrate was loaded onto the HPLC with a YMC ODS-H80 C18 column (S-4 μm, 80 A; 250×10 mm) and the elution by 1% acetonitrile in H_2O with flow rate of 0.5 mL/min were checked with UV monitor at 254 nm. Three peaks appeared at retention times within 15~20 min and each peak was collected, lyophilized, and injected into a mass spectrometer (5989-MS-Engine Machine, HP, USA) .

Mass-spectroscopy

It was performed by direct inlet of the sample to 5989-MS-Engine machine (HP, USA). Ionization was achieved by an electron impact (70eV).

Results and Discussion

It has been well-known as purine salvage pathways. That hypoxanthine-guanine phosphoribosyltransferase (HGPRTase) can catalyze the transfer of the 5-phosphoribosyl group from alfa-D-5-phosphoribosyl 1-pyrophosphate (PRPP) to guanine forming the nucleotide GMP.

$$\text{Guanine (Gua)} + \text{PRPP} \xrightarrow{\text{HGPRTase}} \text{GMP} + \text{PPi} \qquad [1]$$

The forward reaction can be followed at 30°C using spectrophotometry. **Figure 1B** line a, the insertion shows no absorption at 253 nm for Gua. The difference in extinction coefficients at about 253 nm between GMP and Gua (5900 mol/L cm^{-1}) can be used as standard assay of GPRTase forward reaction[6] . The same condition was used when 8-OHGua was placed in the position of Gua for this reaction as the following:

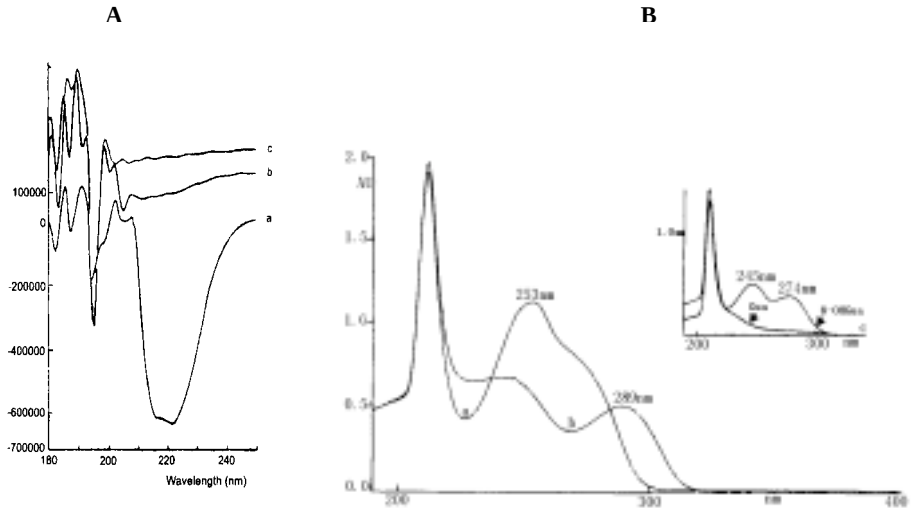
$$\text{8-hydroxyguanine (8-OHGua)} + \text{PRPP} \xrightarrow{\text{HGPRTase}} \text{[8-OH]GMP} + \text{PPi} \qquad [2]$$

Figure 1B, line b shows significant differences from line a. The absorbance peak

at the long wavelength appears at 289 nm, while in the insertion, absorption at 274 nm was observed for 8-OHGua. The CD studies of HGPRTase-product shows that α-helices of HGPRTase is reduced significantly upon the completion of the reaction (**Figure 1A**), which implied that HGPRTase catalyzed both reaction [1] and reaction [2]. Mass analysis for purifying the products in reaction [2] in HPLC gives the identification for the peak with the retention time of 20 min, which is corresponding to [8-OH]GMP *m/z*:379 [M-1] (**Figure 2)**. All the results obtained here clearly prove that the purine salvage pathway can be also applied to 8-OHGua and to form 8-OHGMP.

The evidences imply that the 8-OHGua has a convenient pathway to metabolize *in vivo* so that it can be used the incorporation of 8-OHGua in DNA, which probably contribute to 8-OHGua′s mutational mechanism.

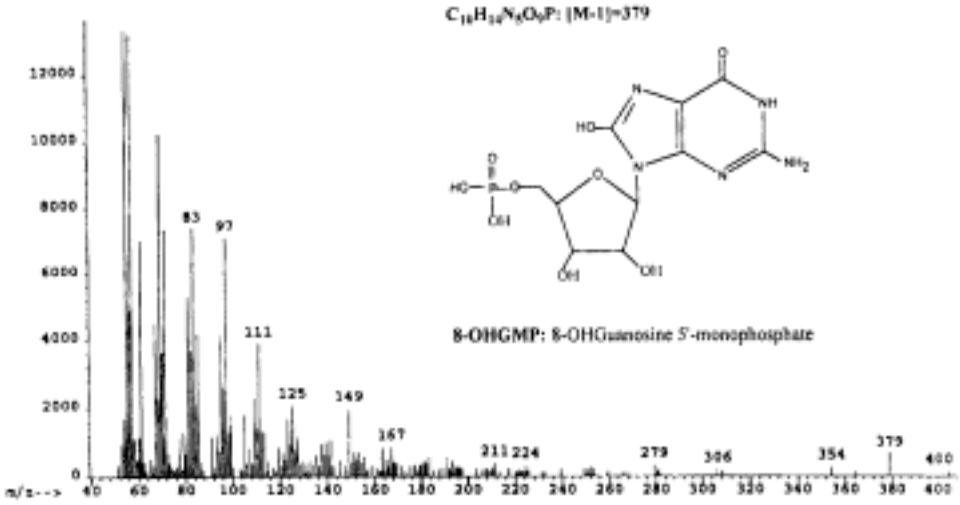
Figure 1 The investigation of 8-OHGua on metabolic mechanism in *vitro*



The conformation characteristics of HGPRTase-product from the reaction of Gua or 8-OHGua and PRPP. a: HGPRTase; b: HGPRTase-product from the reaction of Gua and PRPP; c: HGPRTase-product from the reaction of 8-OHGua and PRPP.
B: The characteristics of spectrophotometic absorbance in the reaction of 8-OHGua and PRPP catalyzed by HGPRTase. a: the reaction of guanine and PRPP catalyzed by HGPRTase; b: the reaction of 8-OHguanine and PRPP catalyzed by HGPRTase.

In conclusion, the 8-OHGua can be incorporated by DNA in *vitro* through the salvage reaction: the reaction of 8-OHGua and PRPP catalyzed HGPRTase and Mg (II). It means that salvage pathway is probably an important metabolic pathway of 8-OHGua.

Figure 2 EI-MS analysis of the fraction from the reaction of 8-OHGua and PRPP catalyzed by HGPRTase. *m/z* 379 is assigned to the [8-OH]GMP



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