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Reduction of Hydroperoxythymines with Glutathione and Glutathione Peroxidase

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Glutathione peroxidase exerted acceleration effect on reduction of cis-6-hydroperoxy-5-hydroxy-5,6-dihydrothymine with glutathione, whereas it exerted little effect on reduction of trans-5-chloro-6-hydroperoxy-5,6-dihydrothymine with glutathione.

Glutathione[1] and glutathione peroxidase[2] are essential for the maintenance of life. On the other hand, ionizing radiation of thymine in aqueous aerated solutions gives a mixture of hydroperoxythymines such as cis-6-hydroperoxy-5-hydroxy-5,6-dihydrothymine (1), its trans-isomer, cis- and trans-5-hydroperoxy-6-hydroxy-5,6-dihydrothymines, and 5-hydroperoxymethyluracil,[3] which are known to have mutagenic activities.[4] Christphersen[5] reported the reduction of a mixture of hydroperoxides formed from the radiation of thymine and DNA with glutathione and glutathione peroxidase. However, little attention has been paid to the reduction of pure hydroperoxythymines. Furthermore, the identification of cis-5,6-dihydroxy-5,6-dihydrothymine (2) and its deoxyribonucleoside in human and rat urine was reported.[6] These observations led us to investigate reduction of 1[7] and trans-5-chloro-6-hydroperoxy-5,6-dihydrothymine (3),[8] which were prepared by the reported methods,[7,8] with glutathione and glutathione peroxidase.

The compounds 1 and 3 were stable in water for more than 3 days. On the other hand, treatment of 1 (20 mg) or 3 (20 mg) with glutathione (40 mg) and glutathione peroxidase (2 mg) in water (10 ml) at room temperature for 30 min gave 2[7] in 95% yield based on 1 consumed (conversion 58%) and trans-5-chloro-6-hydroxy-5,6-dihydrothymine (4)[8] in 98% yield based on 3 consumed (conversion 62%), respectively. Therefore, we examined the time course of the reduction of 1 and 3 in D_2O by comparison of the 5-methyl groups of 1 (δ 1.47, s), 2 (δ 1.40, s), 3 (δ 1.90, s), and 4 (δ 1.81, s) with NMR spectroscopy. This is a new method for analyses of the enzyme reaction.[1,2] The results are shown in Figs. 1 and 2. Figure 1 suggested that glutathione peroxidase served effectively for the reduction of 1 with glutathione. On the other hand, the reduction of 3 with glutathione proceeded readily in the absence of glutathione peroxidase and the enzyme had little effect on the reduction of 3 (Fig. 2).

Since glutathione peroxidase is a selenium containing enzyme,[2] reaction of 1 or 3 with SeO_2 was examined. However, almost no reaction occurred on treatment of 1 (10 mg) or 3 (10 mg) with SeO_2 (10 mg) in D_2O (0.6 ml) at room temperature for 1 h.

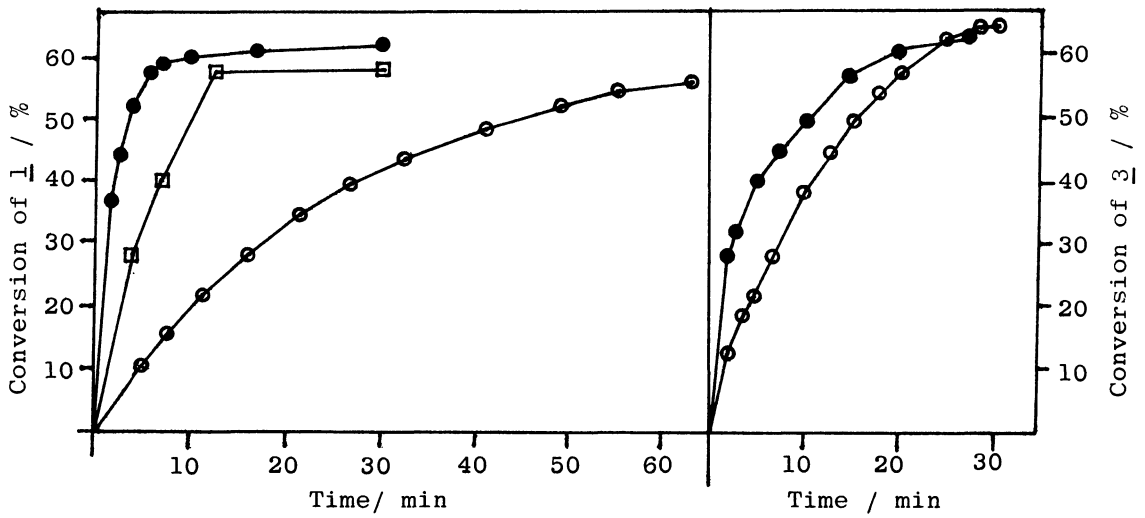
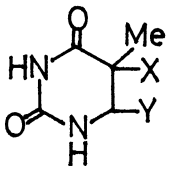


Fig. 1. Reduction of 1 with Glutathione and Glutathione Peroxidase in Deuterium Oxide. Conditions: 1 (12 mg), Glutathione (20 mg), Glutathione Peroxidase, 50 u/mg (Grade III) from Toyobo, (○ None, □ 0.4 mg, and ● 2.3 mg) in D_2O (0.6 ml) at room temperature.

Fig. 2. Reduction of 3 with Glutathione and Glutathione Peroxidase in Deuterium Oxide. Conditions: 3 (12 mg), Glutathione (20 mg), Glutathione Peroxidase (○ None and ● 1.2 mg) in D_2O (0.6 ml) at room temperature.



1 : X=OH, Y=OOH, cis
2 : X=OH, Y=OH, cis
3 : X=Cl, Y=OOH, trans
4 : X=Cl, Y=OH, trans

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7) The structure of 2 was determined by comparison of the authentic sample prepared from oxidation of thymine with $KMnO_4$.[9] The compound 1 was prepared in 40 % yield by treatment of 2[9] with H_2O_2 in 0.1 mol dm^{-3} HCl at room temperature for 24 h according to the manner described by Hahn and Wang.[3b]

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