Radical Reaction of Food Constituents

Photolysis of Uracil in the Presence of Hydrogen Peroxide

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Hydrogen peroxide is used as a bleaching agent for foods containing fish-pastes and pressed-barleys with the procedures such as immersion or spraying using 3~5 percent aqueous solution. However, it has been known that some hydrogen peroxide remains intact in foods depending on the variation of cooking procedures. According to the Food Sanitation Law in Japan, 30 ppm of hydrogen peroxide is the maximum permissible concentration for fish-pastes, and 100 ppm for noodles.

In addition, it was demonstrated that pyrimidine bases (0.1 mM) did undergo rapid, selective and light-dependent decomposition by the ultraviolet irradiation in the presence of a small amount of hydrogen peroxide (3~10 ppm), while purine bases were comparatively stable to the photolysis and furthermore could protect the D-ribose moiety, free or bound, from the photolysis under the same condition. Therefore it is important to investigate the photochemical or radical reaction of food constituents in the presence of hydrogen peroxide and especially to detect the compounds produced by the photolysis from the viewpoint of food chemistry. This paper reports the investigation about photolysis products of uracil in the presence of a small amount of hydrogen peroxide as well as the reaction pathway.

Aqueous solution of uracil (0.1 mM) containing uracil-2-^14C (40.6 mCi/mM, Radiochemical Center, Amersham, England) was irradiated in the presence of 10 ppm hydrogen peroxide (3 eq.) with low pressure mercury resonance lamps for a desired time. The dose at 254 nm was $8.40 \times 10^5$ erg/cm$^2$/min. The absorption maximum at 260 nm of the uracil was lost within 30 min as shown in Fig. 1. The samples irradiated for 10, 20, 30, 60 and 120 min, respectively, were lyophilyzed and quantitatively spotted on Toyo Roshi No. 51 filter paper, which was then developed with the solvent, ethyl acetate-pyridine-water (2:1:2 by volume). The products were analyzed by scanning the chromatograms with the use of 2$\pi$-gas flow counter (Aloka FC-1E). The obtained radioactive spots were identified by the comparison with authentic specimens. The radiochromatograms obtained were shown in Fig. 2. Parabanic acid (Rf 0.76), oxaluric acid (Rf 0.18) and urea (Rf 0.36) were identified. An unidentified spot was found at Rf 0.45 on the chromatogram of the 30 min irradiated sample. The products shown above were also determined by the chromatography using the following solvent system: butanol-acetic acid-water (4:1:5), butanol-water (86:14) and water saturated phenol. No radioactive spot corresponding to barbituric acid or iso-barbituric acid could be detected in any chromatograms. The complete photolysis of uracil,
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FIG. 1. Time Course of Photochemical Conversion of Uracil (0.1 mM, 260 nm, O—O), Barbituric Acid (0.1 mM, 257 nm, △—△) and Parabanic Acid (0.2 mM, 230 nm, △—△) in the Presence of 3 eq. of Hydrogen Peroxide (10, 10 and 20 ppm, respectively).

FIG. 2. Radiochromatograms of the Photolysis Products of Uracil-2-14C in the Presence of Hydrogen Peroxide Irradiated for (A) 10 min, (B) 20 min, (C) 30 min, and (D) 60 min. Details are described in the text.

Based upon the present investigation it is concluded that uracil was decomposed by the ultraviolet irradiation in the presence of hydrogen peroxide to give stable parabanic acid with concurrent formation of less stable oxaluric acid, which was readily hydrolyzed to oxalic acid and urea. Since the uracil solutions were optically dense at 260 nm, virtually most of the incident light was absorbed by uracil. Thus the formation of photoexcited irradiated for 120 min, resulted in the production of parabanic acid (41%), oxaluric acid (21%) and urea (18%) as final products.

Figure 1 shows the photochemical behaviors of barbituric acid, uracil (0.1 mM, respectively) and parabanic acid (0.2 mM) irradiated with the UV-light in the presence of 3 equivalents of hydrogen peroxide. The absorption maximum of barbituric acid was lost more quickly than that of uracil, while parabanic acid was rather stable under the same condition. In addition, it was shown in one experiment that most of parabanic acid-2-14C remained intact only to give a bit of urea after 60 min irradiation. Oxaluric acid has a property to yield urea and oxalic acid by auto-catalyzed hydrolysis in the aqueous condition employed.
uracil triplet should be the initiating step of the reaction. The uracil triplet can effect H atom abstraction from hydrogen peroxide to give the preferred hydrogen addition radical (I), which may react with -OOH radical to yield instable peroxide compound (II). The resulting peroxide is supposed to give rise to barbituric acid (III) under dehydration. On the other hand, the diradical character of the uracil C₆ double bond in the first excited state (IV) suggests that double hydroxylation on the double bond by -OH radicals generated from hydrogen peroxide on UV-irradiation would be possible. A. Kornberg et al., demonstrated that the resulting glycol compound (V) rapidly converted to iso-barbituric acid (VI) under dehydration. Consequently, barbituric acid or iso-barbituric acid would be the intermediate between uracil and the two photolysis products, parabanic acid and oxaluric acid. Due to its very short lifetime under the photochemical conditions employed, however, barbituric acid or iso-barbituric acid must be lost without any accumulation to give parabanic acid in one way and oxaluric acid in another way. Most of urea should be resulted from the hydrolysis of oxaluric acid and no or little from parabanic acid. Radioactive recovery attained 80 percent in this experiment, suggesting that the possible pathway of the reaction shown in chart must be the main.

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