Synergistic Action of Ultraviolet Radiation and Hydrogen Peroxide on Citrulline

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Citrulline/Ultraviolet radiation/Hydrogen peroxide/Photodegradation

Ultraviolet irradiation of citrulline in the presence of hydrogen peroxide formed aspartic acid, glutamic acid, glycine, α-aminobutyric acid, norvaline, arginine and two unidentified ninhydrin-reactive products. Irradiation of citrulline, both in the presence and absence of hydrogen peroxide, formed ammonia and urea. The reactions followed the first-order kinetics with increased reaction constants in the presence of hydrogen peroxide. Without UV light, hydrogen peroxide itself had no effect on citrulline.

Ionizing radiation produces hydrogen peroxide in oxygenated aqueous medium and forms hydroxyl radicals by radiolysis of water1). Peroxide per se has been shown to be nontoxic2) whereas its products OH and other radicals are the most powerful oxygen species known in living organisms3). Thus the aqueous solution of amino acids formed a variety of products when exposed to ionizing radiation due to the presence of oxidizing species, whereas UV light was much less destructive both in terms of number and variety of degradation products4). Rapid photodegradation of thiobencarb5) and phenylalanine6) in the presence of hydrogen peroxide has been reported. The present communication describes the effect of UV light on aqueous solutions of citrulline in the presence of hydrogen peroxide.

The ultraviolet source was a 125 W medium pressure mercury arc bulb without glass envelope (Philips, India), which emits predominantly at 254 nm. Aqueous solutions of citrulline, adjusted to pH 7.0, were exposed to the UV light as described earlier7). The concentration of hydrogen peroxide was 50 mM unless mentioned otherwise. Ion exchange chromatography on Dowex 50W-X8 resin8) was the basic method used in the separation of citrulline-hydrogen peroxide photoproducts. The details of the procedure has been reported earlier6). Amino acids were estimated with the modified ninhydrin reagent9). Ammonia was determined with Nessler’s reagent as described earlier10). For the estimation of urea, the solution was incubated with urease7) and the ammonia liberated was es-

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mated with diacetyl monoxime\textsuperscript{11}). Absorbance was read at 470 nm for urea and 490 nm in case of citrulline.

Citrulline when UV irradiated without hydrogen peroxide did not form any ninhydrin-reactive products. Ion-exchange chromatography of the irradiation products of citrulline (5 mM) in the presence of hydrogen peroxide revealed the formation of aspartic acid, glutamic acid, glycine, $\alpha$-aminobutyric acid, norvaline and two unidentified ninhydrin-positive substances on a long column (Fig. 1) and ammonia and arginine on a short column (Fig. 2). Paper chromatography and thin layer chromatography on silica gel supported these results (data not shown).

Ammonia was formed as a result of UV irradiation of citrulline. Its formation was considerably increased in the presence of hydrogen peroxide (Fig. 3). The formation of ammonia from citrulline at different radiation doses followed the first-order rate kinetics (Fig. 4). A 3.5 fold increase in the rate constant was observed with different concentrations of citrulline in the presence of hydrogen peroxide (Table 1). The formation was, however, dependent on the concentra-

![Graph](image)

Fig. 1. Dowex 50W-X8 chromatography on a long column of the photoproducts from citrulline in a hydrogen peroxide solution. Seven ml aqueous solution of citrulline (5 mM) containing hydrogen peroxide (50 mM) were irradiated with UV light for two hours. The products were chromatographed on a Dowex 50W-X8 column of 0.9 cm x 100 cm length, under the conditions described in the figure. The peaks identified from left to right are aspartic acid, glutamic acid, glycine, $\alpha$-aminobutyric acid and norvaline. The last two peaks were not identified.
Fig. 2. Separation of basic amino acids from UV irradiated citrulline-hydrogen peroxide solution by Dowex 50W-X8 column chromatography. The mixture irradiated for two hours was separated on a 0.9 cm x 15 cm column at room temperature. The experimental conditions were as described in the figure.

...tion of hydrogen peroxide, the rate kinetics is of first order (Fig. 5).

Urea was the other major product of citrulline photodegradation in the presence of hydrogen peroxide (Fig. 6). In the absence of hydrogen peroxide, the amount of urea formed was negligible. The rate kinetics of the formation of urea is of first-order. Apparent rate constants (Table 2) increased two-fold in the presence of hydrogen peroxide. Hydrogen peroxide without UV light was ineffective in the formation of these products from citrulline.

Hydrogen peroxide is known to be formed in the autoxidation of caffeic acid\(^{12}\), irradiated tryptophan solution\(^{13}\), visible light-induced oxidation of ascorbic acid\(^{14}\) and a variety of compounds exposed to the light\(^{15,16}\). Superoxide radical is generated when peroxide is photolysed by near UV radiation\(^{17}\). In a number of other interacting systems the oxidizing species have been shown to be hydroxyl radical\(^{18}\). In the present studies the synergistic action of UV light and hydrogen peroxide resulted in the formation of a number of amino acids even when the individual agents had no effect. Ammonia formation may be due to the deamination of the α-amino group as indicated by intact ureido group when estimated with diacetyl monoxime. The formation of urea could be on the basis of H\(^-\) abstraction by OH\(^-\) attack from C-5 of citrulline molecule.
ACKNOWLEDGEMENT

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Fig. 3. Ammonia formation by UV photolysis of citrulline in the presence (——) and absence (—) of hydrogen peroxide. The concentrations of citrulline are shown in the figure.
REFERENCES


![Graph](image)

Fig. 4. Kinetic plot for the formation of ammonia on UV irradiation of citrulline. Y is the percent change in ammonia liberation at maximum radiation time and Yt is the percent change at any desired time. The concentration of citrulline was 1 mM ( ), 2 mM ( ) and 5 mM ( ) with ( - - - ) and without ( - - - ) hydrogen peroxide.

<table>
<thead>
<tr>
<th>Citrulline concentration</th>
<th>Rate constants (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without peroxide</td>
</tr>
<tr>
<td>1 mM</td>
<td>0.41 x 10$^{-2}$</td>
</tr>
<tr>
<td>2 mM</td>
<td>0.37 x 10$^{-2}$</td>
</tr>
<tr>
<td>5 mM</td>
<td>0.39 x 10$^{-2}$</td>
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*Data were taken from Fig. 4.*

Fig. 5. Effect of different concentration of hydrogen peroxide on the formation of ammonia from UV irradiated citrulline. The concentration of citrulline was 1 mM (—○—) and 5 mM (—■—). Inset-kinetic plot.

Fig. 6. Formation of urea by UV irradiation of citrulline. The concentration of citrulline with hydrogen peroxide was 1 mM (—△—), 2 mM (—●—) and 5 mM (—▲—) and without hydrogen peroxide 5 mM (—○—). Urea was estimated as ammonia after digestion with urease.
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Table 2. Rate constants for the formation of urea from UV irradiated citrulline

<table>
<thead>
<tr>
<th>Citrulline concentration</th>
<th>Rate constants (min⁻¹)</th>
<th>Without peroxide</th>
<th>With peroxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM</td>
<td>NR²)</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>2 mM</td>
<td>NR</td>
<td>1.03 x 10⁻²</td>
<td>1.06 x 10⁻²</td>
</tr>
<tr>
<td>5 mM</td>
<td>0.5 x 10⁻²</td>
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1) Data were taken from Fig. 5.
2) NR = No reaction.

15. W. M. Draper (1979) Ph. D. Dissertation, University of California, Davis, California, USA.