***Experentia*, 11(9), 348- 349, (1955)**
The Action of Hydrogen Peroxide on Amino Acids in Presence of Iron Salts and It's bearing
on Photolysis of Amino Acids, G. D. Kalyankar, C. S. Vaidyanathan, and K. V. Giri

The Action of Hydrogen Peroxide on Amino Acids in Presence of Iron Salts and its Bearing on Photolysis of Amino Acids

Earlier studies[1] on the action of FENTON's reagent on amino acids showed that they are deaminated and converted to aldehydes and corresponding carboxylic acids. Recently JOHNSON *et al.*[2] have shown that α-keto acids are formed by the action of FENTON's reagent on α-amino acids. They have further pointed out that certain enzymatic processes can be simulated by reactions involving free radicals *in vitro*. Therefore the importance of the study of the action of FENTON's reagent on the amino acids is obvious.

We have observed during our experiments that the amino acids undergo a series of complicated changes by the action of hydrogen peroxide in presence of iron salts. A typical experiment carried out to study the action of FENTON's reagent was as follows.

To 0·2 cm³ of 0·1 *M* solution of amino acid was added dropwise 0·2 cm³ of 0·1 *M* ferrous sulphate solution and the volume was made up to 1·8 cm³ with distilled water. To this 0·2 cm³ of H_2O_2 (0·1 *M*) was added and the tube shaken well for about 3 min. Controls were also kept with H_2O_2 alone and also with ferrous sulphate in absence of hydrogen peroxide. After vigorous shaking, the tubes

---

[1] H. D. DAKIN, J. Biol. Chem. *1*, 171 (1905). – C. NEUBERG, Biochem. Z. *20*, 531 (1909). – H. WIELAND and W. FRANKE, Ann. Chem. *457*, 1 (1927).

[2] G. R. A. JOHNSON, G. SCHOLES, and J. WEISS, Science *114*, 412 (1951).

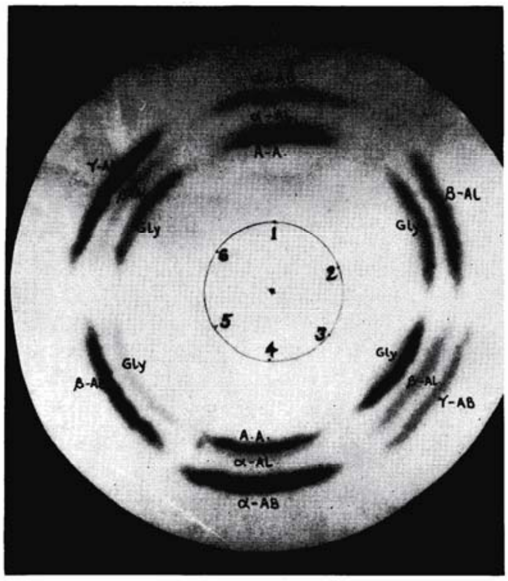

Fig. 1.–Chromatogram showing the action of hydrogen peroxide on amino acids in presence of ferrous and ferric salts (Temperature 24–25° C).
(1) α-aminobutyric acid + H_2O_2 + $FeSO_4$
(2) β-alanine + H_2O_2 + $FeSO_4$
(3) γ-aminobutyric acid + H_2O_2 + $FeSO_4$
(4) α-aminobutyric acid + H_2O_2 + $FeCl_3$
(5) β-alanine + H_2O_2 + $FeCl_3$
(6) γ-aminobutyric acid + H_2O_2 + $FeCl_3$
(α-AB = α-aminobutyric acid, β-Al = β-Alanine, α-Al = α-Alanine, γ-AB = γ-aminobutyric acid, A.A = Aspartic acid, Gly = glycine)

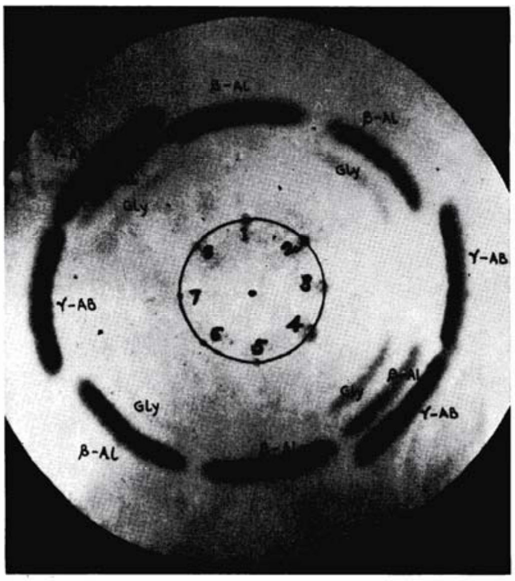

Fig. 2.–Chromatogram showing the photodegradation of amino acids.
(1) β-alanine + $FeCl_3$ in dark
(2) β-alanine + $FeCl_3$ in sunlight
(3) γ-aminobutyric acid + $FeCl_3$ in dark
(4) γ-aminobutyric acid + $FeCl_3$ in sunlight
(5) β-alanine + $FeSO_4$ in dark
(6) β-alanine + $FeSO_4$ in sunlight
(7) γ-aminobutyric acid + $FeSO_4$ in dark
(8) γ-aminobutyric acid + $FeSO_4$ in sunlight
(β-Al = β-alanine, γ-AB = γ-aminobutyric acid, Gly = glycine).

were kept for about 10–15 min at room temperature (24–25° C) and the reaction mixtures were examined for the presence of amino acids by two-dimensional paper chromatography (solvents: butanol-acetic acid-water, 40:10:50 followed by either water-saturated phenol or pyridine-water, 80:20) and also by the circular paper chromatographic technique. The interference by iron salts was overcome by exposing the spot to liquor-ammonia when the iron complex was rendered more mobile and moved with the solvent front (solvent: butanol-acetic acid-water). The chromatogram showing the degradation of amino acids by H_2O_2 in presence of ferrous and ferric salts is given in Figure 1. The amino acids studied were α- and β-alanine. α-amino butyric acid, γ-amino butyric acid, aspartic and glutamic acids and serine. The products formed were the same irrespective of whether ferrous or ferric salts were used. β-Alanine and serine gave glycine; γ-amino butyric acid in turn gave β-alanine and glycine, Glutamic acid gave rise to only aspartic acid, whereas in the case of α-alanine and aspartic acid, ninhydrin-positive products could not be detected. From α-amino butyric acid, the formation of aspartic acid and α-alanine was observed. The amino acids were stable in presence of H_2O_2 alone at room temperature.

It is well-known that when ferrous and ferric salts are added to H_2O_2, it goes through a series of reactions giving rise to free radicals[1]. We have found that the amino acids are degraded by FENTON's reagent only when the molar concentration of ferrous salts is equal to or less than that of H_2O_2. The absence of reaction when the molar concentration of ferrous ions was greater than that of H_2O_2 may be explained by the fact that in this case the regeneration of free radicals by chain mechanism is restricted[1].

The reactions reported here have a striking resemblance to the photolysis of amino acids in presence of TiO_2[2] and to the action of H_2O_2 at 100° C[3]. Considering the close similarity between the action of FENTON's reagent and the photosensitized action of TiO_2 on amino acids, it can be suggested that during the photolysis of amino acids by sunlight in presence of TiO_2, H atoms and OH radicals are generated by dissociation of the water molecule and that the OH radicals are responsible for the oxidative reactions reported earlier[2].

While studying the photolysis of amino acids, it was observed by us that in addition to metal oxides like TiO_2 and ZnO, ferrous sulphate and ferric chloride also acted as efficient photosensitizers and gave the same type of degradative reactions with some of the amino acids, namely, γ-amino butyric acid and β-alanine (Figure 2).

Further studies on the role of these photosensitizers in the oxidative degradation of amino acids are in progress.

G. D. KALYANKAR,
C. S. VAIDYANATHAN, and K. V. GIRI

Department of Biochemistry, Indian Institute of Science, Bangalore 3, India, January 17, 1955.

---

[1] F. HABER and J. WEISS, Proc. Roy. Soc. [*A*] *147*, 332 (1934). – W. G. BARB, J. H. BAXENDALE, P. GEORGE, and K. R. HARGRAVE, Trans. Faraday Soc. *47*, 462, 591 (1951).

[1] W. G. BARB, J. H. BAXENDALE, P. GEORGE, and K. R. HARGRAVE, Trans. Faraday Soc. *47*, 462, 591 (1951).

[2] K. V. GIRI, G. D. KALYANKAR, and C. S. VAIDYANATHAN, Naturwissenschaften *40*, 440 (1953); *41*, 88 (1954).

[3] Y. MATSUO, Nature *171*, 1021 (1953).