Radical Behavior Produced in -Irradiated Alanine and Phenylalanine Amino Acids Aqueous Solutions

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ABSTRACT

Radiolysis of alanine and phenylalanine in aqueous solutions containing NADH has been carried out at different gas saturation and pH. It has been found that the oxidation of NADH decreases by increasing pH. Also, it has been observed that the yield of NADH oxidation in N_2-saturated aqueous solutions is higher than that observed in oxygenated irradiated solution. It is concluded that more oxidizing radicals are generated through the deamination process as a result of the electron addition on the ammo acid. The results also have shown that the yield of NADH oxidation is higher slightly in the case of alanine as compared to that in the case of phenylalanine.

.   NADH

NADH   NADH

NADH   .

.

INTRODUCTION

The radiation chemistry of amino acids has been investigated by many workers (Garrison, 1968; Armstrong and Swallow 1969; Neta and Fessenden, 1970; Fraggi and Tal, 1975). Radiolysis of aqueous solutions of amino acids is considered as one of the important studies in the field of radiobiology. Reaction of water active species, solvated electrons, hydroxyl radicals and hydrogen atoms with amino acids have been postulated (Armstrong and Swallow, 1969). It has been found that deamination of amino acids is the main process that can occur upon the reaction with solvated electrons (Neta and

Fessenden 1970; Fraggi and Tal 1975). In the absence of oxygen. Hydroxyl radicals can either abstract hydrogen atom from ammo acids residues or proceeds via an addition reaction where ever the conditions are available(Armstrong and Swallow, 1969).

In systems where oxygen is present, the radiolysis leads to form at ion of peroxides as hydrogen peroxide and organic peroxidic materials, as intermediates acting as precuser for many final stable products (A) (Fricke et al. 1927; Garrison, 1968; Land and Swallow, 1971).

EXPERIMENTAL

A- materials

All reagents used are of the highest purity available. They were used without further purification.

The following chemicals were obtained from Sigma Chemical Ltd. Alanine (Al), phenylalanine and, the reduced forms of nicotinamide adenine dinucleotide (NADH).

B- procedure

Solutions are made up in deionized water, pH was adjusted with NaOH and phosphate buffer and measured on D8120 Welheim pH 410, pH meter.

Irradiations are carried out with steady state ^{60}Co γ-rays. The dose rate employed is 1.8×10^{17} eV cm^{-3} min^{-1} Gamma cell-220 supplied from atomic energy Canada Limited.

The observed dose was determined by me Fricke-dosimeter (Frike and Hart, 1935) developed by (Miller, 1950).

Spectrophotometric measurements are carried out by using shimadzu UV- Visible recording spectrophotometer, UV 160.

Determination of Peroxides

Peroxides (H_2O_2 and Organic peroxide materials) are measured using iodide method. The method based on that of Alien et al. (1952). Catalaset is(10^{-7}mol dm^{-3}) added to destroy H_2O_2 leaving organic peroxide to be measured by the iodide method.
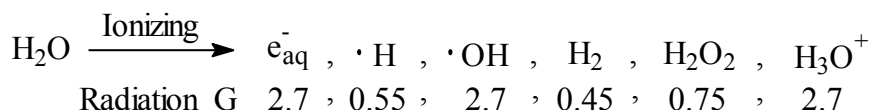
RESULTS AND DISCUSSION

Oxidation of NADH upon γ-irradiation of their N_2 and O_2 saturated aqueous solutions (containing 2×10^{-3} mol dm^{-3} amino acids + 2×10^{-5} or 2×10^{-6} mol dm^{-3} NADH) have been studied under various conditions. The loss of NADH, G(-NADH) is followed spectrophotometrically by the decrease in optical absorption (OD) at 340 nm under the conditions of N_2-saturation. An NAD dimer ($(NAD)_2$) can be formed (Land and Swallow, 1971) and this also has an absorption at 340 nm. The observed change in OD at 340 nm of irradiated N_2-saturated solutions is therefore a direct measure of the loss of NADH and corrections have to be made for dimer formation.

Figure (1) shows the spectra of both NADH and (NAD), taken from the literature(12) and this was repeated in this work and found to be identical. At 400 nm only (NAD_2) absorbs so the yield of dimer can be calculated from the extinction coefficient at this wavelength and correction can then be made for absorbance changes at 340 nm, according to:
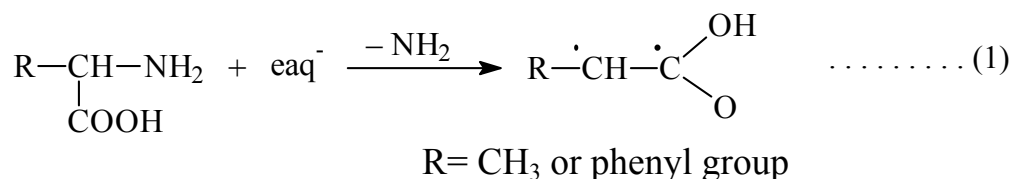
$$OD(corr) = OD(obs) + OD \frac{\varepsilon 340nm(dimer)}{\varepsilon 400nm(dimer)}$$

In order to explain the results listed in Tables (1 and 2), it is very necessary to show the possibilities of reaction of active species (OH·, e_{aq}^- and H) produced on γ-irradiolysis of water) with solutes used. Action of ionization radiation on water can be summarized as in the following (Scholes, 1983).

$$H_2O \xrightarrow{\text{Ionizing}} e_{aq}^- \ , \ \cdot H \ , \ \cdot OH \ , \ H_2 \ , \ H_2O_2 \ , \ H_3O^+$$
Radiation G   2.7 ، 0.55 ،   2.7 ، 0.45 ،   0.75 ،   2.7

The species, OH·, e_{aq}^-, and H· react with amino acid molecules in absence of oxygen. In oxygenated solutions, oxygen efficiently scavenges both H' and <u>solvated electrons</u> (Draganic and Draganic, 1971) leaving OH radical to react with amino acids.

Reaction of solvated electron with amino acids has been studied (Fraggi and Tal, 1975). Alcohols are added to amino acid solution to scavenge H· and ·OH leaving solvated electron to react only with amino acid. The studies show that e_{aq}^- added to carbonyl group. Thus the reactions of e_{aq}^- with amino acids (alanine & phenylalanine amino acids) can be written as:

$$R\!-\!\overset{|}{\underset{COOH}{CH}}\!-\!NH_2 \ + \ eaq^- \xrightarrow{\ -NH_2\ } R\!-\!\overset{\cdot}{C}H\!-\!\overset{\cdot}{C}\!\!\overset{\displaystyle OH}{\underset{\displaystyle O}{\big\langle}} \qquad \cdots\cdots\cdots (1)$$
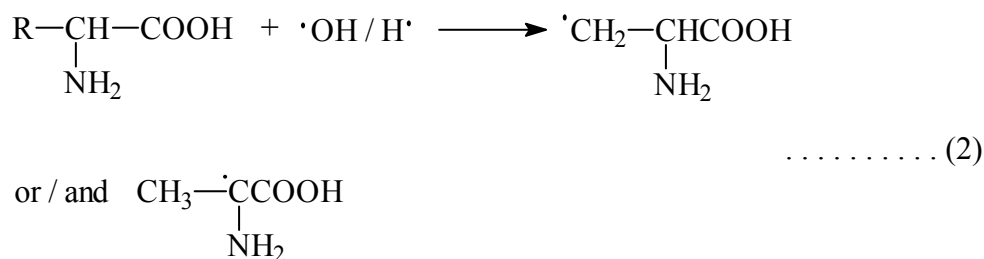
R= CH$_3$ or phenyl group

OH radicals can extract hydrogen atom from aliphatic chain of amino acids (Bennett and Jarrism, 1959; Czapski, 1971). In the case of phenylalanine, addition of OH radicals at the benzene ring can occur under both acidic and neutral conditions (Saeed, 1992).

Oxidation of NADH in the y-radiolysis of alanine and phenylalanine amino acids in both oxygen-and nitrogen-saturated solutions gives an evidence of formation an on these amino acids.

Results in Table 1 show that the oxidation extent of NADH in N$_2$-saturated solutions increases by increasing NADH concentration which reflects that the oxidation process is in competition with other possible reactions involving these radicals, for example the bimolecular reaction between radicals. However, the relatively high G-value of NADH oxidation at pH6 (G 3.2) leads to conclude that radicals formed by electron attack can be converted to oxidizing radicals through deamination process as shown in equation (1).

Abstraction of H atoms by OH and H radicals is expected to occur from β-carbon atom rather under neutral and basic condition from α-carbon atom. (Saeed, 1992), as illustrated in equation 2.

$$R-\underset{\underset{NH_2}{|}}{CH}-COOH \; + \; \cdot OH \,/\, H \cdot \longrightarrow \; \cdot CH_2-\underset{\underset{NH_2}{|}}{CH}COOH$$

$$\cdots\cdots\cdots (2)$$

$$\text{or / and} \quad CH_3-\underset{\underset{NH_2}{|}}{\overset{\cdot}{C}}COOH$$

The radicals formed are expected to be different in their redox behavior; this property accelerates a bimolecular reactions involving these radicals in a competition with the reaction involving NADH oxidation.

Under alkaline condition, the extremely low yield of oxidation of NADH obtained from γ-irradiated amino acid solutions reflect the high repulsion between NADH and amino acid.

In the case of phenylalanine, the direction of ·OH radical has been suggested to occur at the phenyl group under acidic and neutral or slightly basic condition (pH9). Thus, two types of radicals can be formed, radicals derived from H-atom abstraction and radicals formed by addition of OH-radical at the phenyl group of phenylalanine. These two types of radicals are formed in addition to the radicals formed as a result of reaction of solvated electron with amino acid in deoxygenated condition. In this case, bimolecular reactions involving these radicals can compete with oxidation process involving NADH.
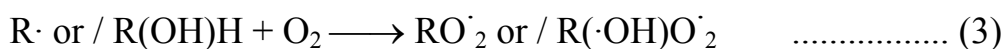
In oxygenated systems, ·OH is the only active species since both H· and solvated electrons are scavenged by oxygen molecule. The radicals formed as a result of OH radicals attack, can react easily with oxygen to give corresponding radicals:
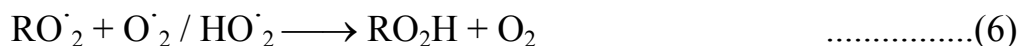
$$R\cdot + O_2 \rightarrow RO_2^{\cdot} \qquad\qquad \cdots\cdots\cdots\cdots (3)$$

The so formed peroxyl radicals can be reduced to hydroperoxide (Scholes and Weiss, 1959) via:

$$R_2^{\cdot} + O_2^{\cdot} / OH_2^{\cdot} \rightarrow RO_2H + O_2 \qquad \cdots\cdots\cdots\cdots (4)$$

The yields of organic peroxidic materials (OP) and H_2O_2 formed in γ-irradiated alanine and phenyl alanine oxygenated solutions were estimated from dose yields plot. Iodide method is used for peroxides determination. Table 2 summarizes the results. However, increasing NADH concentration up to 7×10^{-5} mol dm^{-3} causes the extent of NADH oxidation to increase Table 1. In view of our results interpretation, the following scheme mechanism can be postulated:
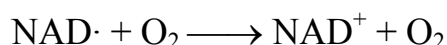
$$RH + OH \longrightarrow R\cdot + H_2O \qquad\qquad \cdots\cdots (1)$$

$$RH + OH \longrightarrow R(\cdot OH)H \qquad\qquad \cdots\cdots (2)$$

$$R\cdot \text{ or } / R(OH)H + O_2 \longrightarrow RO_2^{\cdot} \text{ or } / R(\cdot OH)O_2^{\cdot} \qquad \cdots\cdots (3)$$

$$2RO_2^{\cdot} \longrightarrow \text{Product} \qquad\qquad \cdots\cdots (4)$$

$$RO_2^{\cdot} \longrightarrow \text{Product} \qquad\qquad \cdots\cdots (5)$$

$$RO_2^{\cdot} + O_2^{\cdot} / HO_2^{\cdot} \longrightarrow RO_2H + O_2 \qquad\qquad ....(6)$$

Similar behavior can be expected in the case of R(OH·). The peroxyl radicals formed in equation 3 can oxidise NADH in competition processes with other possible reaction:

$$RO_2^{\cdot} + NADH \longrightarrow RO_2H + NAD\cdot$$
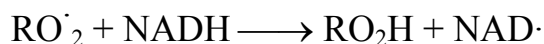
In presence of oxygen, NAD' radical is converted to NAD^+

$$NAD\cdot + O_2 \longrightarrow NAD^+ + O_2$$

The conclusion which can be drawn from this work is the possible formation of oxidizing radicals on ammo acids when biological system is attacked by ionizing radiation, the formed oxidizing radicals can oxidize the most important coenzyme (NADH, NADPH) in intercellular biological system.

Table 1: Oxidation of NADH in γ-irradiated aqueous solutions of alanine and phenylalanine using dose rate of 1.75×10^{17} eV.cm^{-3} at different pH.

G(-NADH)						Gas	
Alanine (10^{-3} M)			Phenylalanine (10^{-3} M)				pH
5×10^{-6} M	2×10^{-5} M	7×10^{-5} M	5×10^{-6} M	2×10^{-5} M	7×10^{-5} M	Flushing	
1.6	2.5	3.2	0.7	2.6	3.1	N$_2$	6
0.86	1.24	2.26	0.4	1.56	2.0	O$_2$	-
1.2	2.2	2.7	1.2	2.0	2.6	N$_2$	7
0.75	1.56	2.0	0.65	1.34	2.3	O$_2$	-
0.8	1.6	2.1	0.43	0.9	1.5	N$_2$	9
0.45	0.9	1.4	0.16	0.44	0.7	O$_2$	-
nil	0.4	0.7	0.3	0.6	1.3	N$_2$	10.4
nil	0.16	0.3	nil	0.2	0.5	O$_2$	-

Table 2: Determination of H_2O_2 organic peroxides (OP) and total peroxide (TP) formed in the irradiation of oxygenated aqueous solutions of alanine and phenylalanine (10^{-3} mol.dm^{-3}) at different pH using the iodide method.

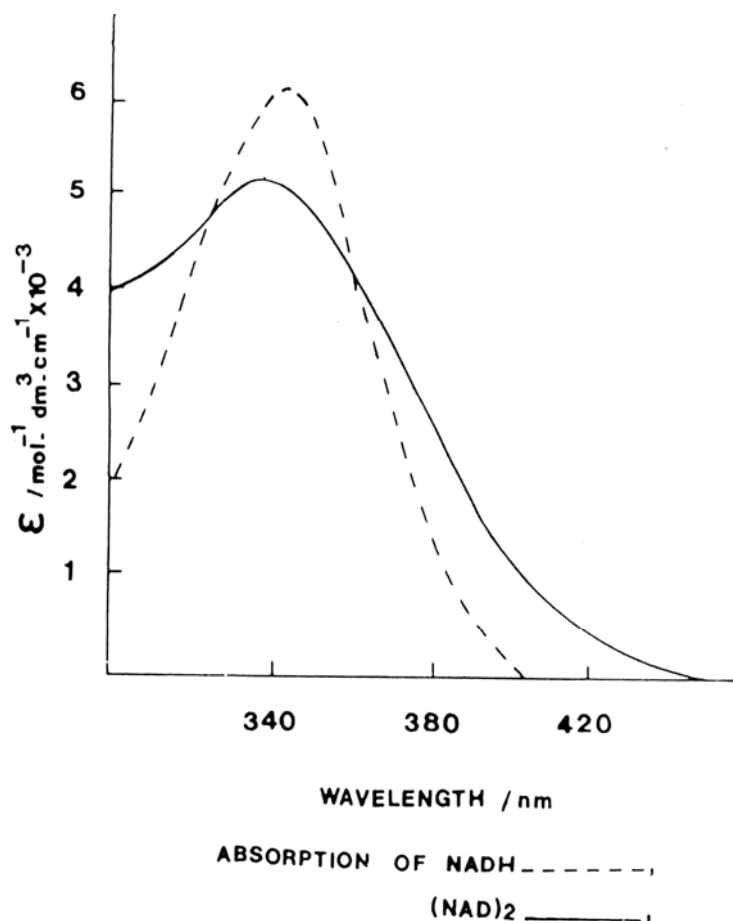|	Alanine			Phenylalanine		
pH	G(H$_2$O$_2$)	G(OP)	G(TP)	G(H$_2$O$_2$)	G(OP)	G(TP)
7	0.8	1.8	2.6	0.7	1.0	1.7
10.4	1.6	2.0	3.6	1.2	2.3	3.5

Fig. 1: Absorption spectrum of NADH and $(NAD)_2$ in aqueous solutions. (Bielski and Chan, 1980).

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