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Effects of γ-ray irradiation on oxidoreductase activities: Yeast alcohol-dehydrogenase and soybean lipoxygenase

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ABSTRACT

The effect of ionizing radiations was known to increase the conservation length of products, to improve their physicochemical properties and/or to enhance the rate of several reactions. The effect of ^{60}Co-gamma-ray irradiation on two oxidoreductase activities was investigated: alcohol-dehydrogenase from yeast and lipoxygenase from soybean. The results showed that the γ-ray doses between 10 and 70 Gy allowed to increase the specific alcohol dehydrogenase activity by 1.45 fold. It was also concluded from the results that the effect of irradiation on alcohol dehydrogenase activity was more important when yeast cells were irradiated before enzyme extraction. In this case, alcohol dehydrogenase activity was enhanced by twelve fold compared to the activity tested in the irradiated enzymatic extract. In contrast, the gamma irradiation doses used to activate the alcohol dehydrogenase did not affect the soybean lipoxygenase activity. Although the belonging of lipoxygenase and alcohol dehydrogenase to the oxidoreductase class, these enzymes have different sensitivities award the gamma ray irradiation.

KEYWORDS

Alcohol-dehydrogenase;
Enzyme activation;
Gamma ray irradiation;
Lipoxygenase;
Saccharomyces cerevisiae.

INTRODUCTION

Radiation chemistry of proteins has been studied for more than 30 years, leading to numerous potential and effective applications to a wide range of food products and medicinal materials[11]. The ionising radiations have been used to increase the conservation length of food products, to modify the chemical properties of some products and/or to enhance the rate of some reactions[5].

The use of enzymes as catalysts in industrial applications is mainly related to their specificity and to their selectivity[8]. Their use is therefore, considered as an alternative way to replace chemical catalysts. In various cases, the activation or the inhibition of enzymes by radiotreatment could be effective and could have an important industrial opening. The effects of radiations on the conformation of enzymes have been the subject

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of several studies[9,10]. Purdie and Lynn[17] have reported that the molecular weight of enzymes was changed as a result of irradiation treatment. Previous studies have also shown that relatively low doses of gamma ray irradiation led to an enhancement of enzyme activities contained in microorganisms[1,2]. Several works described the treatment by irradiation of fruits and vegetables[3,4,12-16,18,21,22] in post-harvest process in order to improve the shelf life of the products, however data specific to the effects on proteins and enzymes is lacking.

Therefore, the main objective of this work is to investigate the impact of ^{60}Co-γ irradiation on enzyme activity. Two oxidoreductases belonging to the lipoxygenase pathway were studied: soybean lipoxygenase (LOX) and alcohol-dehydrogenase (ADH) contained in *Saccharomyces cerevisiae*. The choice of these enzymes was related to their known high sensitivities and their poor stabilities[20]. The LOX catalyses oxidation of polyunsaturated fatty acids producing hydroperoxy-fatty acids; precursors of C6 aroma compounds. The ADH catalyses reduction of aldehydes to their corresponding alcohols. Aldehydes and alcohols have specific aroma properties[6].

MATERIALS AND METHODS

Irradiation

The LOX and ADH activities were tested at different gamma-ray irradiation doses depending on their radio resistance. Two forms of ADH preparations were tested. On one hand, the ADH extracted from dry baker's yeast cells (*S. cerevisiae* purchased from the local market) was treated by different irradiation doses going from 10 to 12500 Gy. On the other hand, the irradiation of dry baker's yeast cells was performed at doses from 10 to 300 Gy. The commercialized solution of lipoxygenase (Sigma, France) was exposed to irradiation doses going from 1.3 to 12.5 kGy. In order to determine the dose rate that could be applied to the preparations, and to have a good distribution of the dose in the irradiated sample in the chosen conditions, cartography has been achieved (D_{max} / D_{min} ratio at 4°C was 1.145 and D_{max} / D_{min} ratio at 25°C was 1.168, Frick dosimeter). The dose rate was 1.77 Gy s^{-1} at 4°C during irradiation of enzyme solution and 1.36 Gy s^{-1} at 25°C during irradiation of dry baker's yeast cells. Irradiation of samples was carried out in the National Center of Nuclear Sciences and Technologies (Tunisia) by using ^{60}*Co* source. The initial activity of the source was about 100.000 curies.

Extraction of yeast alcohol-dehydrogenase

The cytosolic enzyme was extracted from 100 mg of dry baker's yeast cells (*S. cerevisiae)*, which was mixed with 5 mL of phosphate buffer 0.1 M pH 7. The mixture was stirred for 5 min with 5 g of glass beads and centrifuged 10 min at 3000 rpm and 4°C. The supernatant was used to test the ADH activity.

Yeast alcohol-dehydrogenase activity

The ADH activity that catalyses the oxidation of ethanol was determined with a spectrophotometer (Beckman DU530; France) at 25°C by measuring the increase of NADH absorbance at 340nm. The molar extraction coefficient of NADH was 6220 cm M^{-1}. The ethanol oxidation activity was performed in phosphate buffer 0.1 M, pH7 by using 0.2 mM NAD (Sigma, France) and 0.1 M ethanol (Prolabo, France). To initiate the reaction, 35 µL of enzyme solution (0.25 mg mL^{-1}) were added to the mixture. One unit of ADH activity was defined as the amount of enzyme required to oxidize or reduce 1 µmol of cofactor per min under the experimental conditions.

Lipoxygenase activity

LOX activity was tested spectrophotometrically at 25°C, by measuring the increase of absorbance at 234 nm due to appearance of conjugated double bonds. The reaction medium (3 mL) contained 180µL of linoleic acid emulsion, 20 µL of enzyme solution, and glycine buffer 0.1M pH9. The linoleic acid emulsion (10 mM) was prepared with 70 mg of linoleic acid (Sigma, France), 70mg of Tween 20 and 0.5 ml of sodium hydroxide solution (0.5N). The volume was adjusted to 25 ml with distilled water. One unit of lipoxygenase activity corresponds to the absorption increment of 0.001 min^{-1}[7]. All experiments were conducted in triplicates and the results were obtained as the average of the experiments under the same conditions.

RESULTS AND DISCUSSION

Effect of γ-ray irradiation on yeast ADH activity

Biological active material such as enzyme can be

inactivated under irradiation. This inactivation is due to the direct and indirect impact of the ray energy which can alter the enzyme conformation[19].

In this study, the ADH extracted from yeast was irradiated with dose rate equal to 1.77 Gy s^{-1}, and the impact of various doses on the enzyme activity was investigated and was compared to the non irradiated one. Figure 1 shows that the relative activity increased until 1.4 when enzymatic extract was irradiated to a dose of 30 Gy by gamma ray. However, when treating the enzyme at doses between 60 and 100 Gy, ADH activity was progressively reduced and then totally inhibited at doses higher than 70 Gy. The loss of the enzymatic activity by higher doses may be due to the alteration of the enzyme structure.

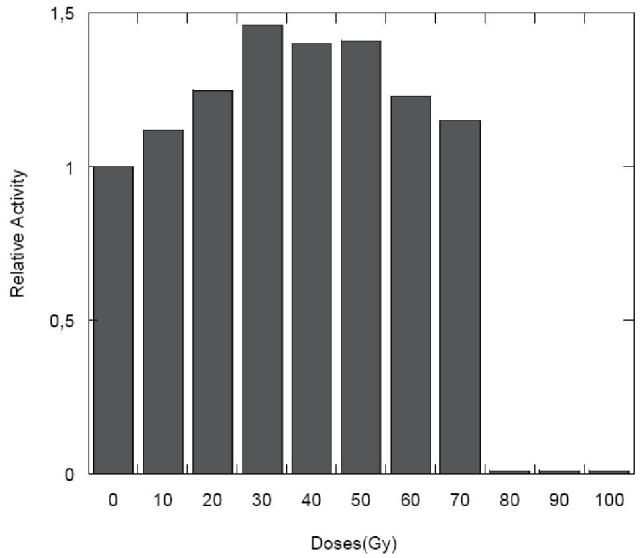

Figure 1 : Effect of different doses of γ-ray on ADH activity from yeast. The dose rate used was 1.77 Gy s^{-1}. ADH activity was measured in phosphate buffer 0.1 M pH 7 in presence of 0.2 mM NAD and 0.1 M ethanol. Activity is relative to the test without irradiation (activity equal to 3.43 U mg^{-1} proteins considered as 1).

Effect of γ-ray irradiation of yeast cells on ADH activity

The ADH activity was extracted from lyophilized yeast cells previously treated with γ-ray irradiation at dose rate equal to 1.36 Gy s^{-1}. A significant increase of ADH activity was observed when cells were treated at doses going from 10 to 300 Gy (Figure 2). The highest ADH activity of 30.95 U mg^{-1} of proteins was obtained after irradiation at 50 Gy. The gamma ray irradiation of yeast cells improved the extracted enzyme activity up to 10 times compared to the non-irradiated sample. The results also suggested that ADH activity contained in irradiated yeast cells stills enhanced at higher γ-ray doses going until 300 Gy compared to the non-irradiated sample. These results are very interesting and demonstrate that the gamma ray irradiation of cells could be considered as efficient and simple method for increasing extracted enzyme activity. They are more spectacular than results obtained after enzyme extract irradiation where ADH activity increases slightly and decreases until total inhibition at 80 Gy (Figure 2). In the latter conditions, free radicals products of radiolysis may act to inactivate the enzyme. So the use of enzyme in its biological environment enhances its protection and radio resistance.

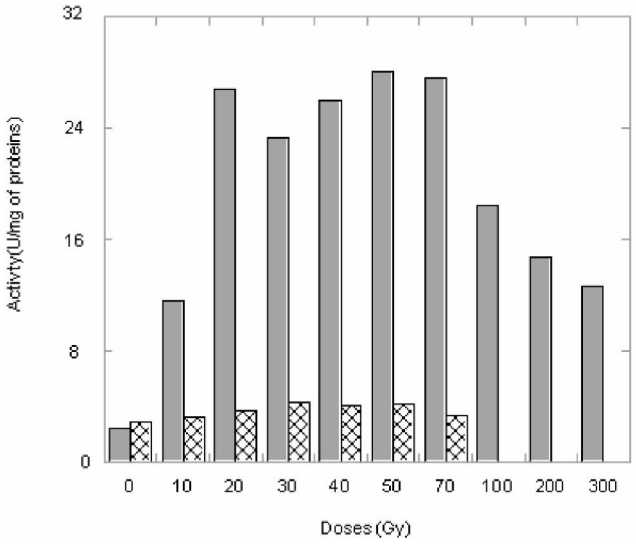

Figure 2 : Effect of ^{60}C gamma ray irradiation of yeast cells or enzyme preparation on ADH activity extracted. Irradiation was carried out at room temperature with a dose rate of (1.36Gy s^{-1}). The activity was measured in phosphate buffer in presence of 0.2 mM NAD (Nicotinamide adenine dinucleotide) and 0.1M of absolute ethanol.

In another series of experiments, the ADH activity was extracted using two methods from yeast irradiated at doses going from 10 to 300 Gy. The enzyme was extracted in presence or in absence of glass beads. The activity was around 3.43 U mg^{-1} of proteins after irradiation and extraction in absence of beads without significant change at different irradiation doses (Figure 3). However the ADH activity extracted by crashing with glass beads increased up to 29.22 U mg^{-1} of proteins after irradiating the yeast cells at 50 Gy. These results demonstrate that the detected ADH was mainly

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intracellular. The fact that ADH activity was detected in spite of absence of glass beads may be explained by a possible effect of the irradiation on the recovery of the enzyme by destabilizing the partition of lipoproteins in yeast cells. The results obtained after irradiation of the whole yeast cells suggest that the yeast cell walls may scavenge radiolytic aqueous free radicals and may protect the enzyme molecules from the radiation-induced denaturation. The ionizing treatment could then replace physical treatments used to liberate enzymes such as the use of glass beads. So, the low gamma ray irradiation doses allowed to increase the ADH activity on one hand, and to permeabilize the yeast cell membranes on the other hand.

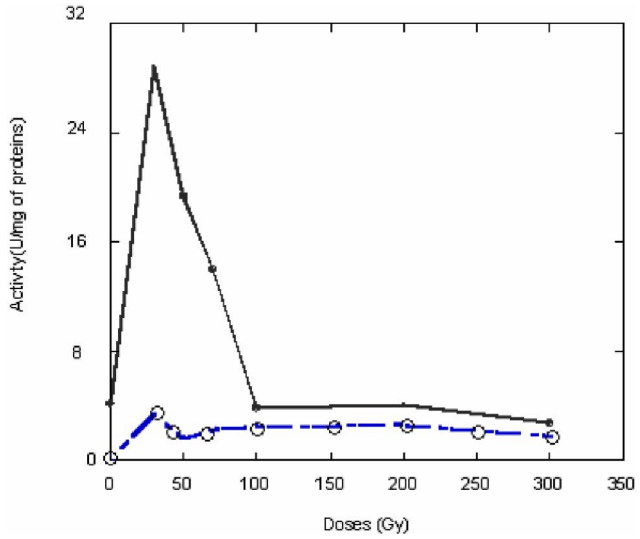

Figure 3 : The effect of gamma ray irradiation on the extraction of ADH from yeast cell. —●— Extraction of ADH with glass beads from yeast irradiated at doses going from 10 to 300 Gy; - -○- - extraction of ADH without glass beads from yeast irradiated at same doses. Irradiation was achieved at room temperature at a dose rate of (1.36 Gy s^{-1}). The activity was measured in phosphate buffer in presence of 0.2 mM NAD and 0.1M ethanol.

Effect of gamma ray irradiation on LOX activity

Tow forms of soybean lipoxygenase have been treated by gamma rays irradiation. Figure 4 shows the effect of irradiation on LOX activity in lyophilized and aqueous forms. A significant difference in enzymatic activity was showed at different doses until 12.5 kGy (Figure 4). The solution of LOX was irradiated, this enzymatic activity was maintained constant and slightly increased at doses between 9 and 12 kGy. In contrast, lyophilized enzyme preparation showed significant increase in the LOX activity as function of gamma ray irradiation doses. In this case, the activity was 6 times higher than the blank test.

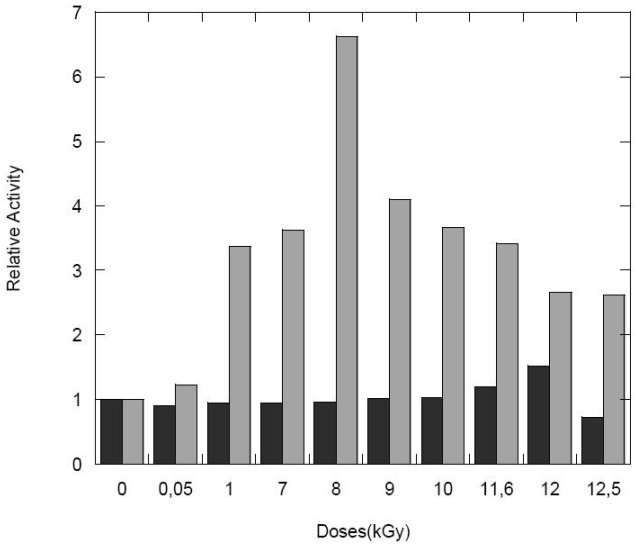

Figure 4 : ^{60}C-γ ray irradiation effect on the enzymatic activity of soybean LOX. The dose rate was of 1.77 Gy s^{-1}. ■ aqueous solution of LOX; ■ lyophilized LOX. The LOX activity was measured in glycine buffer pH9 0.1 M in presence of linoleic acid (10 mM).

As described above, during the irradiation under the aqueous conditions, it may be considered that water-derived radiolytic radicals act to destabilize the enzyme and then to decrease its activity in the aqueous solution of LOX. As shown in figures 2 and 4, enhanced enzymatic activities of LOX and ADH were not affected by the same level of irradiation, suggesting that the radio sensitivity of these two enzymes is so different although they are belonging to the same enzyme class (oxidoreductases).

CONCLUSION

The main objective of this study was to evaluate the influence of gamma (Cobalt 60) radiance dose on the activities of ADH and LOX. The results showed that ADH extracted from yeast was activated by using low irradiation doses of gamma ray (10-70 Gy). Also, a destruction of the cytoplasmic partition by gamma radiances seems to facilitate the extraction of this enzyme. However, the results demonstrated that LOX was activated by higher doses going up to 8 kGy in dry state and 12 kGy in aqueous solution.

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