SHORT COMMUNICATION

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The effect of gamma-radiation on bilirubin

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The effect of gamma-radiations on bilirubin, in vitro, has been studied. It was found that gamma-radiation causes oxidation of bilirubin to biliverdine as one of the products. The likely implication of this effect in transformation of bilirubin to excretable products, in vivo, in case of jaundice is discussed.

Introduction

Normal human metabolism generates some 300 mg per day of bilirubin per individual through the daily breakdown of about 10^{11} red blood cells.[1,2] Bilirubin is present in blood of normal humans in very small amounts (0.2–1.2 mg/100 ml).[3] If the bilirubin content is abnormally high the pigment diffuses through the capillaries and gives the skin and mucus surfaces the characteristic yellow appearance of jaundice. This may be brought about by several causes including: (1) excessive haemolysis, (2) hindrance of excretion due to injury of the polygonal cells of the liver which excrete the pigment, and (3) prevention of excretion due to obstruction of the bile duct (a form of obstructive jaundice).

Oxidation of bilirubin affords biliverdine and other pigments. Bilirubin is insoluble in aqueous medium whereas at physiological pH biliverdine is soluble.[4] It is desirable, in case of jaundice, to convert bilirubin in vivo into the soluble products so that they may be easily excreted. Currently, this is being achieved by in vivo transformation of bilirubin into excretable products[5] by intravenous infusion of sugars or by photo-oxidation of bilirubin.[6–10] The American Academy of Pediatric's guidelines do not support the use of intravenous fluids with jaundiced infants to reduce bilirubin.[11] Photo-oxidation of bilirubin is slow and inefficient, as it has to compete with two faster reactions in which only the shape of the bilirubin molecule is changed. It is, therefore, desirable to develop more safe and effective techniques to transform bilirubin into excretable products. We thought that bilirubin could be broken down to soluble products by gamma-irradiation of the pigment. Generally, molecules with tendency to loose hydrogen, as is the bilirubin molecule, are more sensitive to gamma-radiation.

In the present study bilirubin was gamma-irradiated in solid as well as in solution form in vitro. The results of our study reveal that gamma-irradiation of bilirubin results in the formation of biliverdine. These results are of interest and open up a new area for radiation chemists where the potential of gamma-radiation in the treatment of jaundice can be explored.

Experimental

Bilirubin was obtained from Sigma Chemical Co. and all other chemicals were of analytical grade. Biliverdine was prepared according to a reported method.[12] All irradiations were carried out in air at room temperature (23±2 °C) under a ^{60}Co source (Gamma Beam 650, AECL, Canada). The absorbed dose was measured using the Fricke dosimeter solution.[13] The dose rate was found to be 30.5 Gy·min^{-1}. Spectrophotometric measurements were performed on the Hitachi 220S spectrophotometer.

Various experiments were carried out as follows:

(1) Six samples of finely powdered bilirubin (approximately 100 mg each) in Pyrex glass ampoules were irradiated at various doses (0–1 kGy), shaken with distilled water (10 ml) and centrifuged. The aqueous extracts (3 ml) thus obtained were taken one by one in 10-mm quartz cells and absorption spectra were recorded in the UV-visible range.

(2) Six sample of finely powdered bilirubin (approximately 100 mg each) were suspended in distilled water (10 ml) and irradiated at various doses (0–1 kGy). The spectra of the aqueous extracts, after centrifugation, were obtained as above.

(3) Six samples of bilirubin (approximately 100 mg each) were dissolved in spectroscopic grade chloroform (10 ml) and the solutions irradiated similarly. To the irradiated solutions distilled water (10 ml) was added and the two layers were separated after vigorous shaking. The spectra of the two layers were obtained separately.

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A sample from the aqueous layer was spotted on a silica gel-coated cellulose acetate TLC plate and the chromatogram was obtained by using chloroform – methanol – water (40: 9: 1) system.

All the measurements were performed on six samples so as to determine the statistical significance.

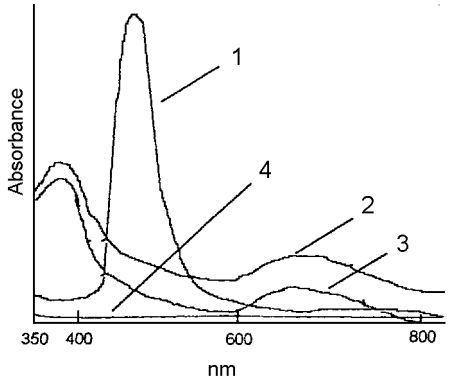

Fig. 1. UV-Visible spectra: bilirubin in chloroform (curve 1), biliverdine in water (curve 2), water extract after gamma-irradiation of bilirubin (curve 3) and water extract without gamma-irradiation of bilirubin (curve 4)

Results and discussion

The spectra of six samples obtained from the experiment 1 at respective doses were reproducible and similar to each other. The representative spectrum is shown in Fig. 1. All the spectra resembled that of the authentic biliverdine in water (Fig. 1) however, the intensities of absorption increased with increasing dose of gamma-radiation. This indicates that gamma-irradiation of solid bilirubin results in the oxidation to biliverdine, which is extractable in water. Similar results were obtained when bilirubin suspended in water was irradiated (experiment 2). These experiments demonstrate that gamma-radiation causes oxidation of the pigment in the solid phase (compare these spectra with that of an un-irradiated sample, treated in the same manner, shown in Fig. 1, curve 4). The effect of γ-radiation was also studied in solution form (experiment 3) using chloroform as solvent. In this case the effect was rapid and pronounced. The spectrum of the water layer resembled that of biliverdine in water.

In the TLC analysis of aqueous extracts from the above experiments two main spots were identified as bilirubin (moved with the solvent front) and biliverdine $R_f = 0.58$; for authentic biliverdine $R_f = 0.58$.

These results show that gamma-radiation causes the oxidation of bilirubin in solid as well as in solution form to produce biliverdine as one of the products. The role of gamma-radiation appears to be to produce free radicals by interacting with the molecular species. The free radicals thus produced are readily converted to the oxidation products. Biliverdine is a non-toxic water-soluble product and is readily excretable without further metabolism.[4] The conversion of bilirubin to biliverdine in vitro by the effect of gamma-radiation can be useful for the transformation of the pigment, in vivo, to excretable products. Further studies are required to establish the potential of the radiation in this regard.

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