Effects of Radiations on Colour of Pearl and on Amino Acid Composition of Conchiolin in Pearl

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After exposure to radiations of γ-rays of Co-60, neutrons of a reactor, and high-speed electrons of a Van de Graaff linear accerelator, pearls of pearl oysters and of fresh water mussels were changed in colour from "white" to "black" of the pearls. The changes were measured by a reflectospectrophotometer, and characterized on an I.C.I. chromaticity diagram.

The remarkable change in colour of the fresh water pearls was attributed to a denatured damage of the proteinous component by γ-irradiation.

Amino acid analysis of the proteinous component of the fresh water pearls was performed by an automatic amino acid analyzer before and after exposure to γ-irradiation.

Radiation sensitivity of amino acids in rigid solid of the pearl was compared with that in free solution and in protein solution, of the previous report.

INTRODUCTION

Pearl is produced by Japanese pearl oyster, *Pinctada martensii*, and fresh water mussel, *Hyriopsis schlegelii*, in pearl culture industry of Japan. The pearl consists of about ninety five per cent of calcium carbonate which is all in an aragonite form, and about five per cent of conchiolin which is a screloprotein. The crystalline form of the calcium carbonate in pearl was characterized to be aragonite[1], and amino acid composition of the conchiolin was reported[2] in the previous papers.

Some changes in colour of the pearls produced by the pearl oyster and the fresh water mussel were observed by Sawada[3] after exposure to irradiation of γ-rays. In the report it was mentioned that any colour change effects, which have been usually observed in some inorganic crystals and glass materials, were not observed in the crystals of the pearls, and the change in colour of the pearls was attributed to some effects on the manganous components of the pearls from the results of an inorganic analysis. However, the remarkable change in colour of the pearls of the fresh water mussel looks like to be due to some denatured damages of the proteinous constituents of the pearls.

The present paper deals with the changes in colour of the pearl after exposure to irradiation of γ-rays, neutrons and high-speed electron beams, and in amino acid composition of the conchiolin in the pearl by γ-irradiation. Radiation sensitivity of amino acids in solution and in protein to γ-rays was reported by the author in the previous paper[4]. Radiation sensitivity of amino acids in conchiolin

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which is contained in rigid solid of the pearl, was discribed and discussed here.

EXPERIMENTAL

Materials. Pearls of the pearl oyster, *Pinctada martensii*, and of the fresh water mussel, *Hiriopsis schlegelii*, were used in these experiments. The pearls of the pearl oyster were cultured in a pearl farm at Ago Bay and the pearls of the fresh water mussel during one or two winters after culture operation.

Irradiation sources: Gamma irradiation was performed by the two-kilocurie cobalt-60 gamma ray irradiation facility[5]. The dose rate was approximately 140 kiloroentgens per hour. Pearls were exposured to γ-irradiation at room temperature.

Neutrons were irradiated in the Japan Research Reactor (JRR-I) of Tokai Laboratory of Japan Atomic Energy Research Institute, Takai-mura.

The high-speed electrons utilized in this experiment were supplied by a Van de Graaff accerelator of The High Voltage Eng. Co., at The Osaka Laboratory of The Japan Association for Radiation Research of High Polymers, Osaka. The dose rate was 540 megaroentgens per hour. All samples of the pearls exposed to the high-speed electrons were irradiated in a crushed ice bath.

Spectroreflectometric measurements: These were performed by a Shimadzu Selfrecording Reflectospectrophotometer Model RC-1 of the Shimadzu Seisakusho Co., Kyoto, according to the method described by Sawada[6] using magnesium oxide back ground.

Amino acid analysis: A KLA-2 Hitachi Amino Acid Analyzer[7] of Hitachi Ltd., Tokyo, was used for an automatic amino acid analysis according to the method described by Moore et al.[8].

Preparation of samples for amino acid analysis: Irradiated pearls were crushed and rendered to hydrolysis in a $6N$ hydrochloric acid solution for 22 hours at 110°C. The resulting hydrolyzate was taken to dryness repeatedly and dissolved in a sodium citrate buffer pH 2.2, for the automatic amino acid analysis.

RESULTS AND DISCUSSION

(1) Effects of radiations on colour of the pearls. Appearance of γ-irradiated pearls of fresh water mussels was changed remarkably to black brown, contrary to that of sea water pearl oysters, which was changed to faint bluish brown. Fig. 1 shows reflectospectra of irradiated pearls of the sea water pearl oysters with γ-rays of Co-60, neutron fluxes of the reactor, and high-speed electron beams of the Van de Graaff linear accerelator. Fig. 2 shows reflectospectra of γ-irradiated pearly of the fresh water mussels. Colour values at three axes, x, y and z of these irradiated pearls were presented in Table 1 and pictured on an I.C.I. chromaticity diagram of Fig. 3. The pearls of the pearl oyster and of the fresh water mussel used in this experiment should be belongs to a white group of the chromaticity diagram of pearls on the I.C.I. chromaticity diagram[9], before exposure to radiations. They were shifted from the white group to a black group of the diagram after exposure to radiations.

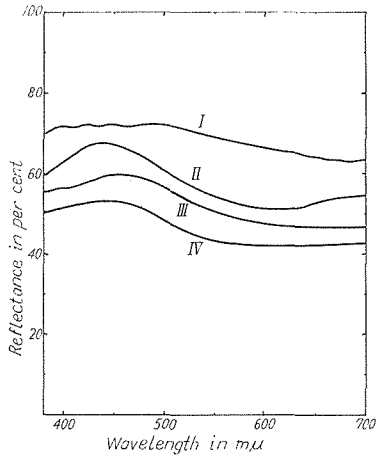

Fig. 1. Reflection curves for irradiated pearls of the pearl oyster.
I: non-iradiated, II: irradiated with a 2.8×10^{16} n/cm^2 dose of neutrons, III: irradiated with a 1.1×10^8 r. dose of γ-rays, IV: irradiated with a 10^8 r. dose of high-speed electrons.

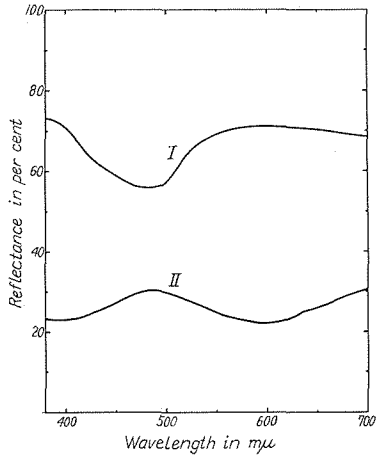

Fig. 2. Reflection curves for irradiated pearls of the fresh water mussel.
I: non-irradiated, II: irradiated with a 10^7 r. dose of γ-rays.

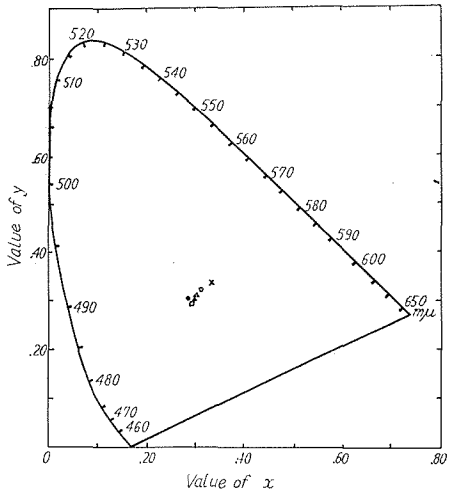

Fig. 3. Colour domain on the C.I.E. colour diagram of the irradiated pearls of the pearl oyster and of the fresh water mussel.
I: non-irradiated sea water pearl ○, II: irradiated with a 10^8 r. dose of γ-rays ●, III: sea water pearl irradiated with a 2.8×10^{16} n/cm^2 dose of neutrons □, IV: sea water pearl irradiated with a 10^8 r. dose of high-speed electrons △, V: non-irradiated fresh water pearl ×, VI: fresh water pearl irradiated with a 10^7 r. dose of γ-rays ▲.

The radio-resistancy of the colour change of the sea water pearls seems to be due to its rigid structure of aragonite and conchiolin. The remarkable change of the colour of the fresh water pearls appears to be some denatured damages of conshiolin of the pearl since the pearls was more rich in proteinous components than the sea water pearls.

Table 1. Three colour values, x, y, and z, of the pearls before and after exposure to irradiation.

Material	Irradiation dose	Colour value		
		x	y	z
Sea water pearl	None	0.3026	0.3122	0.3852
Sea water pearl	γ-Rays 1.1×10^8 r.	0.2952	0.3046	0.4010
Sea water pearl	Neutrons 2.8×10^{16}n/cm^2	0.2926	0.2961	0.4113
Sea water pearl	Electrons 10^8 r.	0.2973	0.2996	0.4031
Fresh water pearl	None	0.3251	0.3280	0.3469
Fresh water pearl	γ-Rays 10^7 r.	0.2967	0.3087	0.3946

It was proposed that the colour changes of γ-irradiated pearls may be attributed to a change in manganous components which were contained in a small amount in the pearls from the results of elementary analysis of inorganic substances. However, colour changes of proteinous substance and amino acid preparation by irradiation of high-speed electron beams in low temperature of solid carbon dioxide or liquid air, were observed preliminary to be distinguishable from the changes in colour center of crystals[10]. The observation may suggest that the change in colour of the pearls is probably due to a damage of the conchiolin of the pearls.

(2) **Effect of γ-irradiation on amino acid composition of the pearls.** Amino acid analyses of non-irradiated and γ-irradiated pearls of the fresh water mussels were performed by the automatic method using the analyzer. The amino

Table 2. Amino acid composition of the pearls before and after exposure to γ-irradiation.

Amino acid	Amount per 100 g. of pearl material		
	Before irradiation	After exposure to 10^7 r. dose	Destruction %
Asp	0.576	0.569	1.2
Thr	0.109	0.107	1.9
Ser	0.385	0.382	0.7
Glu	0.272	0.242	11.0
Pro	0.032	0.029	9.3
Gly	0.851	0.830	2.5
Ala	1.020	1.015	0.4
Val	0.149	0.146	2.1
Met	0.024	0.020	16.6
Ileu	0.108	0.101	6.5
Leu	0.322	0.317	1.6
Tyr	0.075	0.072	4.0
Phe	0.245	0.238	2.7
His	0.330	0.224	32.0
Arg	0.282	0.280	0.6
NH_3	0.740	0.925	(25.0*)

* liberation pre cent of ammonia.

Table 3. Scope of radiation sensitivity of amino acids to gamma radiation under various conditions.

Dose of γ-rays in r.	37% survival after exposure to γ-radiation				
	Free amino acid in solution[11,4]	Free amino acid in solid[12]	Amino acid residue of protein in solution[13,4]	Amino acid residue of protein in solid[14]*	Amino acid residue of protein in pearl
10^5	Met, Thr, Leu, Phe, His, Arg, Glu, Ser, Ileu, Lys, Val, CyS, Tyr, Gly, Asp,		Tyr, Phe, Arg, His, Thr, Ileu, Leu, Lys, Gly, Asp, Glu, Ala,		
10^6	$CySO_3H$ Ala, Pro, Tyr,		Ser, Pro, Val,		
10^7					His, Met,
				Ileu,	
10^8		Tyr, Arg, Cys, Asp, Ileu, Ala, Met, Lys,		Arg, Leu, Lys, His, Val, Met, Phe, Gly,	Glu, Pro, Ileu, Tyr,

* under an anaerobic condition.

acid composition determined from the results was presented in Table 2.

It was found that in the pearl histidine residue of the conchiolin was most radiosensitive after exposure to 10^7 r. dose of γ-rays. The radiation caused 32 per cent destruction of the histidine residue, 16.6 per cent of the methionine, 11.0 per cent of glutamic acid, and 9.3 per cent of proline, in the protein of the pearl. However, arginine and serine which had been found to be radiosensitive in protein and free amino acid, in the previous papers and results[11~14], were radioresistant to the gamma irradiation in this experiment.

A scope of radiation sensitivity of amino acids to radiation doses of γ-rays under the conditions of free in solution, of free in solid, of amino acid residue of protein in solution and of amino acid residue of protein in solid, was presented in Table 3. Some protecting effects to the radiation was observed in the rigid structure of the pearl.

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