Effect of Irradiation on the Carbohydrate Metabolism Responsible for Sucrose Accumulation in Potatoes

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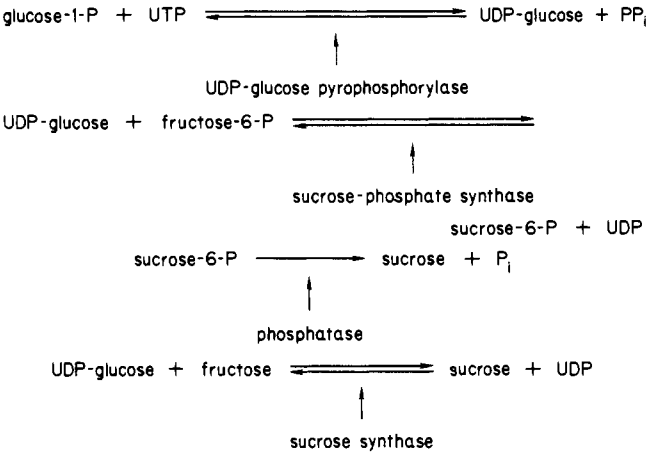
The aim of this work was to investigate the effect of γ-irradiation on the carbohydrate metabolism relating to the accumulation of sucrose in irradiated potato tissues. [^{14}C]Sucrose, UDP-glucose, fructose, or fructose 6-phosphate was administered to tissues followed by incubation for 10 h at 20 °C, and then the distribution of ^{14}C was determined. Irradiation restricted the breakdown of sucrose and accelerated its synthesis especially from UDP-glucose and fructose 6-phosphate. It was suggested that sucrose-phosphate synthase with an enhanced activity played an important role in the sucrose accumulation in irradiated potatoes. The radioactivity of CO_2 was higher and that of water-insoluble substances was lower in irradiated tissues as compared with unirradiated ones. The effect of irradiation could not be observed in tissues pretreated with cycloheximide, which indicated the synthesis of protein was responsible for these phenomena.

γ-Irradiation has been reported to increase the sucrose content of potato tubers (Becker and Somogyi, 1977; Becker et al., 1979; Burton, 1975; Burton et al., 1959; Hayashi and Kawashima, 1982a, 1983; Jaarma, 1967; Schwimmer et al., 1957), sweet potato roots (Hayashi and Kawashima, 1982a,b), and chestnuts (Hayashi et al., 1983). The level of sucrose once enhanced by irradiation did not decrease during storage after irradiation, when the samples were irradiated at a higher dose (Hayashi and Kawashima, 1982b, 1983; Hayashi et al., 1983). The sucrose content was dependent upon irradiation dose, and the maximum sucrose content was obtained at 2–3 kGy in potato tubers (Hayashi and Kawashima, 1982a). The sucrose accumulation was accompanied by the decrease in starch content in irradiated sweet potato roots, which suggested that γ-irradiation accelerated the conversion of starch into sucrose (Hayashi and Kawashima, 1982a). The sucrose contents of irradiated vegetables and fruits increased for a long period after irradiation (Hayashi and Kawashima, 1982a,b, 1983; Hayashi et al., 1983). For example, the sucrose content continued to increase for 1–2 weeks after irradiation in irradiated potatoes (Hayashi and Kawashima, 1982a, 1983) and 3–4 weeks in irradiated sweet potatoes (Hayashi and Kawashima, 1982b). These results suggested that the sucrose accumulation was not caused by direct chemical reactions but by physiological reactions.

Studies on the mechanism of this sucrose increase have been done by determining the activities of enzymes of sugar metabolism in irradiated potato tubers, and the enhancement of the activities of phosphorylase (Becker and Somogyi, 1977; Hayashi and Kawashima, 1983; Schwimmer et al., 1958; Ussuf and Nair, 1972), UDP-glucose pyrophosphorylase (Hayashi and Kawashima, 1983), sucrose synthase (Becker et al., 1979; Hayashi and Kawashima, 1983; Hayashi et al., 1984; Jaarma, 1966b), and sucrose-phosphate synthase (Hayashi and Kawashima, 1983; Hayashi et al., 1984) has been observed so far. The activities of sucrose synthase and sucrose-phosphate synthase were enhanced when sweet potato roots were irradiated (Hayashi et al., 1984). It was suggested that the acceleration of sucrose synthesis and starch degradation due to these enzymes with enhanced activities resulted in the accumulation of sucrose in irradiated potato tubers and sweet potato roots. These results were obtained by determining the enzyme activities of the protein fraction extracted from potato tubers (Hayashi and Kawashima, 1983; Hayashi et al., 1984) or sweet potato roots (Hayashi et al., 1984), and the extraction procedure might have influenced the enzymes and their inhibitors and activators.

We felt it was worth investigating the effect of irradiation on the metabolism responsible for the synthesis and breakdown of sucrose in sliced tissues (disks) of potato tuber by using radiolabeled compounds in order to elucidate the mechanism of sucrose accumulation in irradiated potato tubers. In this study [^{14}C]sucrose, UDP-glucose, fructose, or fructose 6-phosphate was supplied to unirradiated or irradiated tissues, and the distribution of radioactivity was determined after incubation for 10 h at 20 °C, with the assumption that sucrose is synthesized through the enzyme reactions of sucrose synthase and sucrose-phosphate synthase as follows.



The administration of the radiolabeled compounds to the tissues was done 24 h after irradiation, because both the rate of the sucrose increase and the activities of the enzymes playing a role in sugar metabolism were at the highest levels in potato tubers 1–2 days after irradiation (Hayashi and Kawashima, 1983).

MATERIALS AND METHODS

Potato. Potatoes of variety Dejima grown in Nagasaki-ken were obtained from a local market in Tsukuba, Ibaraki-ken.

Irradiation of Potato. Potato tubers were irradiated with a Gamma Cell 220 (Canada AEC, 6.2 kGy/h) at 2 kGy in ambient atmosphere. The accuracy of the irradiation dose was within ±15%.

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Preparation of Disks. Potato tubers were washed with sterilized deionized water, and then disks (1 × 12 mm) were prepared followed by washing with sterilized potassium phosphate buffer (50 mM, pH 6.5). One disk weighed around 0.12 g.

Determination of Sucrose Content. The disks were prepared immediately after irradiation and then incubated at 20 °C with 1 mL of 50 mM phosphate buffer (pH 6.5) in a sterilized Petri dish. The sucrose content of the disks was determined with eight disks at regular intervals during storage according to Hayashi and Kawashima (1982a).

Determination of Respiratory Rate. Eight disks prepared immediately after irradiation were placed in a sterilized Erlenmeyer flask (100 mL) with an airtight rubber seal and incubated with 1 mL of 50 mM phosphate buffer (pH 6.5) at 20 °C. One milliliter of the air inside was analyzed by gas chromatography to determine O_2 and CO_2 by using a Shimadzu GC-3AH (Shimadzu Seisakusho Co.) equipped with a thermoconductivity detector and a 0.3 × 200 cm Wg-100 column (Gasukurokogyo Co.). The chromatograph was run isothermally at 70 °C, and Ar at a flow rate of 40 mL/min was used as the carrier gas.

Incubation of Disks with a Labeled Compound. [U-^{14}C]Sucrose (Amersham International, 350 Ci/mol), [U-^{14}C]UDP-glucose (Amersham International, 200 Ci/mol), [U-^{14}C]fructose (Amersham International, 300 Ci/mol), and [U-^{14}C]fructose 6-phosphate (Centre D'etudes Nucleaires des Salay, 250 Ci/mol) were used in this study as labeled compounds. Eight disks prepared 24 h after irradiation were placed with 1 mL of 50 mM phosphate buffer (pH 6.5, sterilized) containing 5 μCi of the radioactive compound in a sealed beaker (50 mL, sterilized) through which an air stream (free from microorganisms) was passed at a flow rate of 5 mL/min and CO_2 evolved by the tissues was absorbed in 5 N KOH. The disks in the beaker were incubated for 10 h at 20 °C.

Pretreatment of Tissues with Cycloheximide. Eight disks prepared immediately after irradiation were incubated in 1 mL of 50 mM phosphate buffer (pH 6.5) with or without 20 μM cycloheximide for 24 h at 20 °C followed by the addition of 5 μCi of [^{14}C]UDP-glucose and then incubated for 10 h at 20 °C.

Extraction and Fractionation of Radioactive Substances. After incubation the disks were washed with water and put into boiling 80% ethanol. The extraction from the disks was carried out successively with the following in the boiling state: 80% ethanol, 50% ethanol, 20% ethanol, water, and finally 80% ethanol again. The residue was called water-insoluble substance. The combined extracts were dried in vacuo followed by the addition of 200 μL of water. The extracts were fractionated by two-dimensional paper chromatography in 1-butanol–acetic acid–H_2O (60:15:25) and methanol–formic acid–H_2O (80:15:5). The radioactive substances on the paper detected by autoradiography were extracted with water and further fractionated by paper chromatography in other solvents, if necessary. The spots with radioactivity on the paper were cut out, and the radioactivity was determined by liquid scintillation spectrometry.

The radioactivity in the water-insoluble substance was determined according to Kojima and Uritani (1972), and that of CO_2 was determined according to Dixon and Rees (1980).

RESULTS AND DISCUSSION

Sucrose Content and Respiratory Rate of Tissues. The sucrose content in the tissues prepared immediately after irradiation continued to increase during storage at 20 °C (Table I). The sucrose content of the irradiated tissues increased by 4.5 times during storage for 3 days after irradiation, while that of irradiated whole tubers increased more than 10 times (Hayashi and Kawashima, 1983), which indicated that the degree of the sucrose increase in the tissues was not so high as when the irradiated potato tubers were stored without cutting. The sucrose content of the unirradiated tissues slightly increased but not so distinctly as that of the irradiated tissues.

Table I. Sucrose Content of Potato Tissues (Percent, Fresh Weight)[a]

dose, kGy	storage period, days 0	1	2	3
0	0.18	0.21	0.21	0.23
2	0.17	0.36	0.57	0.78

[a] Each value is a mean of four measurements from four potato tubers.

Table II. Respiratory Rate of Potato Tissues (μg of CO_2 Evolved by 1 g of Tissues for 1 min)[a]

dose, kGy	storage period, days 0	1	2	3
0	2.80	2.83	2.77	2.81
2	3.22	6.48	5.77	4.57

[a] Each value is a mean of four measurements from four potato tubers.

Table III. Distribution of ^{14}C after Metabolism of [^{14}C]Sucrose by Potato Tissues for 10 h at 20 °C (Percent)[a]

fraction	unirradiated	irradiated
CO_2	1.8	6.1
water-insoluble substances	8.3	2.0
phosphate esters	2.8	1.0
sucrose	63.0	84.3
hexoses	10.0	3.6
UDP-glucose, ADP-glucose	1.2	0.8
others	12.9	2.2
total count, dpm	1.96×10^6	1.76×10^6

[a] Each value is a mean of four measurements from four potato tubers. The potato tubers were stored at 20 °C for 24 h after irradiation, and the disks were prepared followed by the immediate addition of the radiolabeled compound.

Irradiation increased the respiratory rate of the potato tissues (Table II), and the respiratory rate reached the maximum 1 day after irradiation and then it decreased, which agreed with the results on irradiated potato tubers (Hayashi and Kawashima, 1983; Ogata et al., 1959), although the degree of the increase in the irradiated tissues (2.3 times 1 day after irradiation) was not so high as that in irradiated potato tubers (6 times 1 day after irradiation; Hayashi and Kawashima, 1983). The evolution of CO_2 of 2.8 μg g^{-1} min^{-1} in the unirradiated tissues was higher as compared with 0.2 μg g^{-1} min^{-1} in unirradiated tubers (Hayashi and Kawashima, 1983), which indicated that slicing caused some physiological changes in potatoes as reported by Rees and Beevers (1960).

However, the increase in sucrose content and respiratory rate of the potato tissues caused by irradiation was so distinct as to show that irradiation influenced the metabolism of sugars and thus justified the use of the tissues in the study on the mechanism of sucrose accumulation in irradiated potatoes.

Distribution of ^{14}C after Metabolism of [^{14}C]Sucrose. The irradiated tissues retained more ^{14}C in sucrose as compared with the unirradiated ones after incubating the tissues with [^{14}C]sucrose for 10 h at 20 °C (Table III), which indicated that irradiation restricted the breakdown of sucrose in potato tissues. Not only invertase but also sucrose synthase has been reported to be responsible for

Table IV. Distribution of ^{14}C after Metabolism of [^{14}C]UDP-glucose by Potato Tissues for 10 h at 20 °C (Percent)[a]

fraction	unirradiated	irradiated
CO_2	6.2	12.3
water-insoluble substances	21.0	3.1
phosphate esters	29.9	21.5
sucrose	7.9	33.0
hexoses	3.1	1.1
UDP-glucose, ADP-glucose	17.5	9.0
others	14.4	20.0
total count, dpm	1.02×10^6	9.18×10^5

[a] Each value is a mean of four measurements from four potato tubers. The potato tubers were stored at 20 °C for 24 h after irradiation, and the disks were prepared followed by the immediate addition of the radiolabeled compound.

Table V. Distribution of ^{14}C after Metabolism of [^{14}C]Fructose by Potato Tissues for 10 h at 20 °C (Percent)[a]

fraction	unirradiated	irradiated
CO_2	3.6	6.1
water-insoluble substances	26.8	12.3
phosphate esters	22.4	38.9
sucrose	10.4	15.0
hexoses	21.6	20.6
UDP-glucose, ADP-glucose	1.9	1.4
others	13.3	5.7
total count, dpm	1.36×10^6	9.18×10^5

[a] Each value is a mean of four measurements from four potato tubers. The potato tubers were stored at 20° for 24 h after irradiation, and the disks were prepared followed by the immediate addition of radiollabled compound.

the cleavage of sucrose in potato tubers and sweet potato roots (Murata, 1971, 1974), but the activities of these two enzymes in potato tubers were not lowered by irradiation (Hayashi and Kawashima, 1983; Hayashi et al., 1984; Jaarma, 1966a,b). Therefore, it is suggested that the changes not directly relating to enzymes like invertase and sucrose synthase played a role in the increased accumulation of sucrose in irradiated potatoes.

Distribution of ^{14}C after Metabolism of [^{14}C]UDP-glucose. The radioactivity of sucrose in the irradiated tissues was 4 times higher than that in the unirradiated ones after metabolizing [^{14}C]UDP-glucose (Table IV), which indicated that the conversion of UDP-glucose into sucrose was accelerated by the irradiation of potato tissues. UDP-glucose and/or ADP-glucose in the irradiated tissues retained less ^{14}C than in the unirradiated ones. The lower level of ^{14}C-labeled UDP-glucose and/or ADP-glucose in the irradiated tissues compared to the unirradiated tissues indicates the enhanced activity of enzymes metabolizing UDP-glucose, which is consistent with the increase in the activities of UDP-glucose pyrophosphorylase, sucrose synthase, and sucrose-phosphate synthase found by Hayashi and Kawashima (1983).

Distribution of ^{14}C after Metabolism of [^{14}C]-Fructose or [^{14}C]Fructose 6-Phosphate. The radioactivity of sucrose was 1.5 times higher in the irradiated tissues than that in the unirradiated tissues after metabolizing [^{14}C]fructose (Table V), and that of sucrose in the irradiated tissues was 2.5 times higher than that in the unirradiated ones after metabolizing [^{14}C]fructose 6-phosphate (Table VI). The radioactivity of sucrose was much higher when [^{14}C]fructose 6-phosphate was metabolized as compared with when [^{14}C]fructose was metabolized. Fructose 6-phosphate would contribute to the synthesis of sucrose in potato tissues to a greater extent than fructose. The results in Tables IV–VI suggest that sucrose phosphate synthase, of which the substrates are UDP-glucose and fructose 6-phosphate, plays a more important

Table VI. Distribution of ^{14}C after Metabolism of [^{14}C]Fructose 6-Phosphate by Potato Tissues for 10 h at 20 °C (Percent)[a]

fraction	unirradiated	irradiated
CO_2	10.8	19.3
water-insoluble substances	23.8	5.3
phosphate esters	24.1	13.0
sucrose	18.1	45.1
hexoses	4.4	10.6
UDP-glucose, ADP-glucose	1.5	1.4
others	7.3	5.3
total count, dpm	1.05×10^6	1.29×10^6

[a] Each value is a mean of four measurements from four potato tubers. The potato tubers were stored at 20 °C for 24 h after irradiation, and the disks were prepared followed by the immediate addition of the radiolabeled compound.

Table VII. Distribution of ^{14}C after Metabolism of [^{14}C]UDP-glucose by Potato Tissues for 10 h at 20 °C without Cycloheximide (Percent)[a]

fraction	unirradiated	irradiated
CO_2	7.9	12.9
water-insoluble substances	35.9	10.9
phosphate esters	14.2	8.2
sucrose	5.8	35.7
hexoses	1.0	1.6
UDP-glucose, ADP-glucose	19.2	4.1
others	16.0	26.6
total count, dpm	1.14×10^6	1.56×10^6

[a] Each value is a mean of four measurements from four potato tubers. The disks were prepared immediately after irradiation followed by the incubation in 50 mM phosphate buffer for 24 h at 20 °C, and then the radiolabeled compound was added.

role in the synthesis of sucrose in irradiated potatoes as compared with sucrose synthase, of which the substrates are UDP-glucose and fructose.

The incorporation of ^{14}C into CO_2 was increased by irradiating tissues, while that into water-insoluble substances was reduced, when any radiolabeled compound that was used in this study was metabolized (Table IV–VI). Higher rate of CO_2 evolution due to an enhanced respiratory rate would be responsible for this higher degree of incorporation of radioactivity into CO_2. The change in the balance of the synthesis and breakdown of starch may be one of the mechanisms of sucrose accumulation in irradiated potatoes, which can be partly explained by the enhancement of the activity of phosphorylase in irradiated potatoes (Becker and Somogyi, 1977; Hayashi and Kawashima, 1983; Schwimmer et al., 1958; Ussuf and Nair, 1972). The effect of irradiation on the activities of enzymes responsible for the synthesis and degradation of starch should be investigated in addition to phosphorylase in order to obtain enough information to clarify the physiological changes relating to starch metabolism in irradiated potatoes.

Effect of Cycloheximide on the Distribution of ^{14}C after Metabolism of [^{14}C]UDP-glucose. The tissues prepared immediately after irradiation showed the same effect of irradiation as the tissues prepared 24 h after irradiation on the incorporation of radioactivity into sucrose, CO_2, and water-insoluble substances when radiolabeled UDP-glucose was metabolized (Table VII). Pretreatment of tissues with cycloheximide removed these effects of irradiation and significantly lowered the radioactivity of CO_2 and water-insoluble substances (Table VIII). These results indicated that protein synthesis played a role in the synthesis of water-insoluble substances and respiration in both of the unirradiated and irradiated tissues. The radioactivity of sucrose in the unirradiated tissues was not influenced by the treatment with cyclo-

Table VIII. Distribution of ^{14}C after Metabolism of [^{14}C]UDP-glucose by Potato Tissues for 10 h at 20 °C with Cycloheximide (Percent)[a]

fraction	unirradiated	irradiated
CO_2	1.4	1.4
water-insoluble substances	3.9	3.6
phosphate esters	43.5	54.6
sucrose	6.3	7.4
hexoses	5.8	8.7
UDP-glucose, ADP-glucose	20.5	8.3
others	18.6	16.0
total count, dpm	1.29×10^6	1.12×10^6

[a] Each value is a mean of four measurements from four potato tubers. The disks were prepared immediately after irradiation followed by the incubation with cycloheximide in 50 mM phosphate buffer for 24 h at 20 °C, and then the radiolabeled compound was added.

heximide, while that in the irradiated tissues was reduced to the level in the unirradiated ones. It is indicated that the accelerated synthesis of sucrose requires protein synthesis in the irradiated potatoes. It is suggested that the enzymes of which the activities are enhanced by irradiation such as sucrose phosphate synthase and sucrose synthase are rapidly synthesized in irradiated potatoes, which is consistent with the results reported by Nair (1969) in that the synthesis of protein (asparagine synthetase) was accelerated in irradiated potato tubers.

CONCLUSION

The results in this study and the previous work (Hayashi and Kawashima, 1983; Hayashi et al., 1984) suggest that sucrose phosphate synthase plays a more important role in the sucrose accumulation in irradiated potatoes as compared with sucrose synthase. The accelerated synthesis of sucrose phosphate synthase is one of the important physiological changes responsible for the accumulation of sucrose in irradiated potatoes. The reduced breakdown of sucrose and the increased degradation of starch also play a role in the sucrose accumulation. It is concluded that γ-irradiation brings about various physiological changes that together contribute to the accumulation of sucrose in irradiated potato tubers.

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Formation of *N*-Nitrosodimethylamine in Korean Seafood Sauce

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Traditional Korean kimchi prepared from several combinations of vegetables and seafood sauces has been examined for the presence of volatile nitrosamines. Except for trace levels in some samples, no nitrosamines were detected in untreated kimchi and, in most cases, only very low levels of *N*-nitrosodimethylamine (NDMA) could be detected following reaction of the kimchi with acidic nitrite. Kimchi prepared with shrimp sauce or anchovy sauce, however, was found to contain parts-per-million levels of NDMA after treatment with acidic nitrite. Virtually all of this nitrosamine appeared to arise from the fermented shrimp sauce or anchovy sauce used in the preparation of the kimchi. Formation of NDMA in fermented shrimp sauce was effectively inhibited by the addition of ascorbic acid prior to treatment with nitrite.

Kimchi, an important traditional food in Korea, is prepared from salted radish or Chinese cabbage by the addition of fermented anchovy or shrimp sauce along with seasonings and spices, e.g., red pepper powder, garlic, or ginger. In some areas fish or oysters are added to bring out the traditional local flavor. Kimchi prepared near Seoul usually contains shrimp sauce while that prepared in more southern areas tends to contain anchovy sauce. A previous investigation, into the changes in composition and properties of kimchi during preparation, led to evidence suggesting that *N*-nitroso compounds might form at some stages during this process (Kim et al., 1984). This evidence included high initial levels of nitrate followed by a gradual decrease in nitrate concentration, along with decreasing pH, decreasing concentrations of ascorbic acid,

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