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Papaya Seed Represents a Rich Source of Biologically Active Isothiocyanate

YOSHIMASA NAKAMURA,*,† MOTOKO YOSHIMOTO,† YOSHIYUKI MURATA,† YASUAKI SHIMOISHI,† YUMI ASAI,‡ EUN YOUNG PARK,‡ KENJI SATO,‡ AND YASUSHI NAKAMURA‡

Department of Biofunctional Chemistry, Division of Bioscience, Graduate School of Natural Science and Technology, Okayama University, Okayama 700-8530, Japan, and Department of Food Sciences and Nutritional Health, Kyoto Prefectural University, Shimogamo, Sakyo-ku, Kyoto 606-8522, Japan

In the present study, papaya (*Carica papaya*) seed and edible pulp were carefully separated and then the contents of benzyl isothiocyanate and the corresponding glucosinolate (benzyl glucosinolate, glucotropaeolin) quantified in each part. The papaya seed with myrosinase inactivation contained >1 mmol of benzyl glucosinolate in 100 g of fresh seed. This content is equivalent to that of Karami daikon (the hottest Japanese white radish) or that of cress. The papaya seed extract also showed a very high activity of myrosinase and, without myrosinase inactivation, produced 460 μmol of benzyl isothiocyanate in 100 g of seed. In contrast, papaya pulp contained an undetectable amount of benzyl glucosinolate and showed no significant myrosinase activity. The *n*-hexane extract of the papaya seed homogenate was highly effective in inhibiting superoxide generation and apoptosis induction in HL-60 cells, the activities of which are comparable to those of authentic benzyl isothiocyanate.

KEYWORDS: Papaya; isothiocyanate; glucosinolate; apoptosis; antioxidant; HL-60

INTRODUCTION

A number of studies support the fact that certain food phytochemicals protect against cancer. An important group of compounds that have this property are organosulfur compounds including isothiocyanates (*1*). Isothiocyanates, naturally occurring in abundance in cruciferous vegetables such as broccoli, watercress, Brussels sprouts, cabbage, Japanese radish, and cauliflower, may play a significant role in affording the cancer chemopreventive activity of these vegetables. They are stored as glucosinolates in plants and are released when the plant tissue is damaged or ground. The chemopreventive properties of cruciferous vegetables might be attributed to their high content of glucosinolates, which are responsible for their pungent odor and biting taste. Indeed, certain glucosinolates (benzyl, *p*-hydroxybenzyl, and 2-hydroxybut-3-enyl glucosinolates) themselves induce phase 2 carcinogen detoxifying enzymes (*1*). The conversion from glucosinolates into isothiocyanates is catalyzed by myrosinase, a thioglucosidase that is physically separated from glucosinolates under normal conditions, but, in the human diet, the myrosinase in cruciferous vegetables is heat-inactivated during cooking. Conversely, glucosinolates may also be hydrolyzed in the intestinal tract, because the microflora possess a myrosinase-like activity (*2*−*4*).

Various isothiocyanates are effective chemoprotective agents against chemical carcinogenesis in experimental animals (*5*−*7*). More recently, several epidemiological studies have indicated that the dietary consumption of isothiocyanates or isothiocyanate-containing foods inversely correlates with the risk of developing lung, breast, and colon cancers (*8*−*10*), providing evidence that they have a potential to prevent cancer in humans. Our group recently focused on the diverse biological activities of isothiocyanate compounds and demonstrated potent inducing effects of phase 2 enzyme and apoptosis by benzyl isothiocyanate (BITC; **1**) isolated from the extract of papaya (*Carica papaya*) whole fruits (*11*−*18*). Although exhibiting a spectrum of biological activities similar to those of sulforaphane and phenethyl isothiocyanate, both of which are representative isothiocyanates in cruciferous plants, **1** is rare in Brassicaceae vegetables (*1*). It should also be noted that **1** has unique potential to inhibit cell growth specifically in proliferating cells (*17*) and to express an antioxidant effect in an electrophilic reaction-dependent manner (*18*). Therefore, papaya fruits have been paid much attention because of their specific content of **1**. It was previously confirmed that BITC is formed from benzyl glucosinolate (**4**; glucotropaeolin) in papaya seeds (*19*). Some papers have shown that **1** was detected in papaya pulp at a much lower level than in the seeds (*20*, *21*), which seems not to exclude the possibility that the wounded papaya seeds or their constituents would be contaminants in the tested preparation of the papaya pulp. In addition, to our knowledge, there are only a few studies that made a direct comparison of the contents

* Author to whom correspondence should be addressed (telephone/fax 81-86-251-8300; e-mail yossan@cc.oakayama-u.ac.jp).
† Okayama University.
‡ Kyoto Prefectural University.

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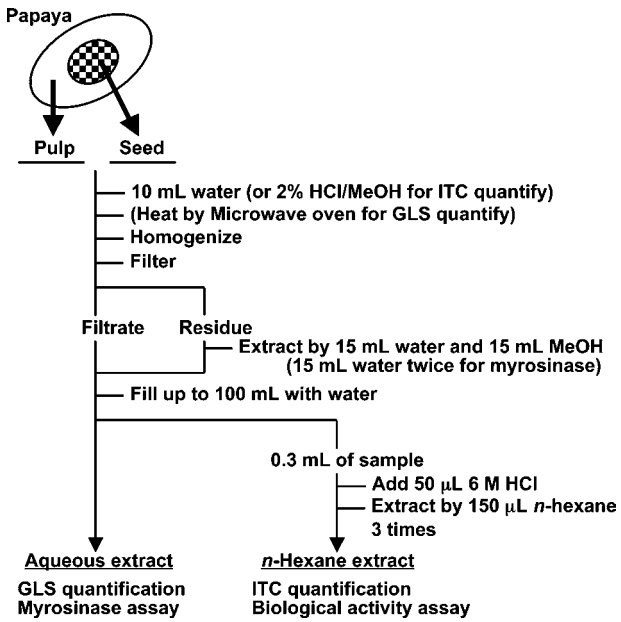

Figure 1. Sample preparation procedure for the papaya extracts.

of **1** and **4** and myrosinase activity between the papaya seed and edible pulp. In this study, we carefully separated the papaya seed and edible pulp without any wounding and then quantified the contents of **1** and **4** in each part. We compared the biological activities, such as superoxide generation inhibition and apoptosis induction induced by *n*-hexane extracts, between the seeds and pulp. This study also provided biological evidence that **1** is the predominant anticarcinogenic agent in the papaya seed, but not in the papaya pulp, and that the papaya seed is a significant source of glucosinolate other than *Brassica* vegetables.

MATERIALS AND METHODS

Chemicals. Allyl isothiocyanate (**2**) and **1** were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Sigma-Aldrich Chemical Co. (St. Louis, MO), respectively. Sinigrin (**5**) was purchased from Acros Organics (Geel, Belgium). 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) was obtained from Research Biochemicals International (Natick, MA). All other chemicals were purchased from Wako Pure Chemical Industries Ltd.

Papaya Samples. Papaya (*C. papaya*, Solo variety, yellow pulp, fully ripened) and Karami daikon (*Raphanus sativus*, a traditional wild strain) were purchased from local supermarkets in Kyoto, Japan, from September through December 2005.

Pereparation for Content of Glucosinolates. The sample preparation procedure is summarized in **Figure 1**. Ten-gram samples (daikon; papaya seeds or pulp) were put into a ceramic mortar with 10 mL of distilled water, covered with plastic wrap, and heated for 3 min in a microwave oven (500 W) for the myrosinase inactivation. The samples were homogenized with a ceramic pestle and then filtered. The residue was separately extracted twice with 15 mL of methanol and distilled water. The filtrate and methanol and water extracts were then combined and made up to 100 mL with water, followed by analysis of the glucosinolates (**4** and 4-methylthio-3-butenyl glucosinolate, **6**) contents using a paired-ion HPLC.

Preparation for Contents of Isothiocyanates and Cell Assays. Ten-gram samples (daikon; papaya seeds or pulp) were put into a ceramic mortar with 10 mL of distilled water or 2% HCl−methanol and homogenized with a ceramic pestle. After a 30-min incubation, the residue was separately extracted twice with 15 mL of methanol and distilled water. The filtrate and methanol and water extracts were then combined and filled to 100 mL with water. Fifty microliters of 6 M HCl was added to 0.3 mL of the extracted sample solution to quench the activity of myrosinase prior to analysis of the contents of isothiocyanates (**1** and 4-methylthio-3-butenyl isothiocyanate, **4**), and the solution was extracted three times each with 150 μL of *n*-hexane. The *n*-hexane extracts were combined, and the combined extract was subjected to the reverse-phase HPLC to measure the content of isothiocyanates in each sample and cell assays to measure the inhibitory activity of superoxide generation and cytotoxicity.

Myrosinase Activity Determination. Ten-gram samples (daikon; papaya seeds or pulp) were placed in a ceramic mortar with 10 mL of distilled water and homogenized with a ceramic pestle and then filtered. The samples were filtered, and the residue was extracted twice with 30 mL of distilled water. The water extracts were combined and made up to 100 mL with water. The activity of myrosinase was determined by the following method. Ten milliliters of the aqueous extract sample solution was centrifuged for 5 min at 7000*g*, and 40 mL of cold acetone kept in a −25 °C freezer was added to the supernatant, followed by incubation for 15 min and centrifugation for 5 min at 7000*g*. The precipitate that appeared was dissolved in 0.5 mL of 33 mM potassium phosphate buffer solution at pH 7.0. Ten microliters of the solution was added to 500 μL of the reaction mixture containing 33 mM potassium phosphate buffer, pH 7.0, 10 mM **5**, 500 μM ascorbic acid, and 1 mM EDTA to initiate the enzymatic reaction; after 3 min, the reaction was quenched by adding 5 μL of a 6 M HCl solution. Compound **2** liberated by the enzymatic reaction was extracted three times with 150 μL of *n*-hexane. The content of **2** in the combined *n*-hexane extract was measured by the reverse-phase HPLC method. One unit of myrosinase activity is equivalent to the hydrolysis rate of **5** at which 1 μmol of **2** per minute can be liberated.

HPLC Analyses of Glucosinolates and Isothiocyanates. The concentrations of the glucosinolates and isothiocyanates were measured using HPLC, Shimadzu (Kyoto, Japan) model LC-10Ai instrument with an SPD-10Ai UV detector and C-R5A integrator. A 250 × 4.6 mm i.d. 5C 18-MS column (Waters) was employed as the analytical column for the glucosinolate analysis using paired-ion chromatography. Ten microliters of an aliquot sample was injected and eluted by a linear gradient from 0 to 70% of acetonitrile in a 5 mM tetra-*n*-butylammonium hydrogen sulfate aqueous solution at 1 mL/min. The UV absorbance at 230 nm was monitored to detect **4**. A 75 × 4.6 mm i.d. ODS-H80 column (YMC, Kyoto, Japan) was used for the **1** and **2** analyses by reverse-phase chromatography. Ten microliters of an aliquot sample was injected and then isocratically eluted at 1 mL/min with 40 and 55% acetonitrile in water containing 0.1% trifluoroacetic acid for **1** and **2**, respectively. The UV absorbance at 254 nm was monitored to detect **1** and **2**. The glucosinolate and isothiocyanate contents were calculated by using a linear standard equation derived from the absorptions of a serial dilution of authentic samples. For the glucosinolate content, **5** was used as a standard because **4** and **6** are not commercially available.

Cell Culture. Acute promyelotic leukemia HL-60 cells (Health Science Research Resources Bank, Osaka, Japan) were maintained in RPMI 1640. The media were supplemented with 10% heat-inactivated fetal calf serum (Trace Scientific, Ltd., Melbourne, Australia), 50 units/mL penicillin, and 50 μg/mL streptomycin and grown in an atmosphere of 95% air and 5% CO_2 at 37 °C.

Superoxide Generation Test in Differentiated HL-60 Cells. The inhibitory tests of the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced superoxide generation in dimethyl sulfoxide (DMSO)-differentiated HL-60 cells were done as previously reported (*14*). Briefly, to determine the inhibitory effect of the superoxide generation, the test compounds dissolved in 5 μL of DMSO were added to a DMSO-induced differentiated HL-60 cell suspension (1×10^6/mL) and incubated at 37 °C for 15 min; 100 nM TPA and cytochrome *c* solution (1 μg/mL) with or without superoxide dismutase (150 units/mL) were added to the reaction mixture, which was incubated for another 15 min. At the end of the incubation period, the cell suspension was transferred to an ice bath. After centrifugation at 250*g*, the visible absorption at 550 nm was measured. The inhibitory effect was expressed by the relative decreasing ratio of absorbance of a test compound to the control experiment.

Assay for Cell Viability. HL-60 cells (1×10^4) in 50 μL of culture medium were mixed with 50 μL of the sample in 96-well microculture plates. After culturing at 37 °C for 24 h, the number of viable cells was determined. For quantitative analysis of cell viability, 100 μL of

Table 1. Level of Glucosinolates, Isothiocyanates, and Myrosinase in Papaya and Karami Daikon[a]

	μmol/100 g			
plant part	glucosinolate[b]	isothiocyanate (HCl +)[c]	isothiocyanate (HCl −)[c]	myrosinase
papaya seed	1269.3 ± 90.0	1.43 ± 0.65	461.4 ± 14.2	4584.0 ± 47.2
papaya pulp	<3.0	<0.3	<0.3	<1.0
daikon (Karami)	1272.3 ± 79.6	nd[d]	421.0 ± 27.2	541.1 ± 29.2
Brussels sprout[e]	265	nd[d]	nd[d,f]	nd[d]

[a] Values are mean ± SD ($n = 3$). [b] Glucosinolates in papaya, daikon, and Brussels sprout are **4**, **6**, and **5**, respectively. [c] Isothiocyanates in papaya, daikon, and Brussels sprout are **1**, **3**, and **2**, respectively. HCl treatment during extraction is for myrosinase inactivation. [d] Not determined. [e] Data from ref *24*. The values are the mean of two separate samples. Brussels sprout also contained 21.9 μmol/100 g of 3-butenyl glucosinolate. [f] Brussels sprout juice contains 2.2 mM 3-butenyl isothiocyanate and no detectable amount of **5**.

the culture medium and 10 μL of an AlamarBlue solution were added to each well, and the fluorescence was measured with excitation at 560 nm and emission at 590 nm according to the manufacturer's directions after incubation at 37 °C for 2 h in a humidified CO_2 incubator. The obtained values were compared with those of the control incubated with vehicle only.

Flow Cytometric Analysis. Cell cycle analysis was performed on the harvested cell pellets treated with 0.2% Triton X-100 in phosphate-buffered saline (PBS) and propiodium iodide (PI) solution (20 μg/mL) containing RNase A (100 μg/mL). The mixture was immediately analyzed by a flow cytometer. The cell cycle distribution was measured using the EPICS XL System II (Beckman Coulter, Tokyo, Japan).

DNA Fragmentation. HL-60 cells were incubated in a culture medium in the presence or absence of papaya seed extract. For the DNA fragmentation analysis, 5×10^5 cells were pelleted by centrifugation, and the DNA was isolated from the cell pellets as described by Sellins and Cohen (*22*). The DNA (20 μg) was then subjected to electrophoresis in 2% agarose gels, stained with ethidium bromide, and then imaged with an LAS3000 image-analyzer (Fuji Film, Tokyo, Japan).

RESULTS AND DISCUSSION

Measurement of 1 and 4 in Papaya. We first determined the aqueous preparations heated for 3 min in a microwave oven for myrosinase inactivation that provided the maximum level of the glucosinolate content. As shown in **Table 1**, **4** in the papaya seeds was 1269 μmol (564 mg)/100 g of fresh seed. This amount in the papaya seed is equal to that in Karami daikon (1272 μmol (579 mg)/100 g), a traditional wild strain of the Japanese white radish exhibiting the hottest taste and most potent antimutagenic activities among the daikon strains (*23*), and much higher than that in Brussels sprouts as previously reported (*24*). Furthermore, a recent review, with the objective of developing a database for the glucosinolate content of cruciferous vegetables from 18 published studies providing 140 estimates for 42 items (*25*), demonstrated that the highest glucosinolate values were for cress (389 mg/100 g), whereas the lowest values were for Pe-tsai Chinese cabbage (20 mg/100 g). These data suggested that papaya seed is an equivalent or richer source of glucosinolate compared to cruciferous vegetables. Next, we measured the amount of **1** in the intact papaya fruit with HCl treatment to inactivate myrosinase. With enzyme inactivation, 100 g of the papaya seed contained only 1.43 μmol (213 μg) of **1**. In contrast, grated seeds without HCl treatment had an increased **1** level of 461 μmol (68.7 mg)/100 g, also comparable to the **3** level in grated Karami daikon [421 μmol (66.9 mg)/100 g]. No other hydrolysis products or hydrolysis product metabolites were detected in the papaya seed preparations. Also, the papaya seed preparation showed as expected a potent hydrolysis activity toward **5** (4600 units/100 g). This activity is much stronger than that in Karami daikon (541 units/100 g). These results suggested that the papaya seed is a better source of myrosinase in addition to an isothiocyanate precursor, glucosinolate. In spite of containg 10 times more myrosinase than Karami daikon, the grated papaya seed contains **1** as much as **3** in the grated Karami daikon, suggesting that **4** might be less sufficient as a myrosinase substrate than **5** or **6**. Contrary to the case of the papaya seeds, the **1** and **4** contents and myrosinase activity in the papaya pulp were below the detection level with or without myrosinase inactivation.

There are several studies demonstrating that the extract of whole papaya fruits contains a significant amount of **1** and shows certain biological effects. A quantitative comparison study of the **1** content of each part of the papaya fruit reported that the papaya pulp contains 21.2−43.1 ppm of **1**, which is about 10 times lower than that in the seed (*21*). An additional consideration that was not evaluated in this study was that there is variability among the papayas not only for the **4** and myrosinase contents but also for ripeness. To the best of our knowledge, the present study is the first publication to quantify the content of glucosinolates in papaya fruits and determine that papaya seeds are an extremely richer source compared to the pulp.

A consideration relevant to the medicinal use of papaya seeds is if **1** might be released from **4** by myrosinase catalysis. A previous paper showed that a significant level of **5** was detected in Brussels sprouts, but **2** was not detected in the Brussels sprout juice (*24*). Conversely, we demonstrated here that a significant amount of **1** was detected in the grated seed aqueous extract, suggesting that in the papaya seed, there might be slight impediments to inhibit the myrosinase activity or to break down the product. Factors that are able to inhibit myrosinase or inactivate **1** could compromise the concentration of **1** in the body. One such factor is acid (pH 2) in the stomach. Although we utilized a very high concentration of HCl (6 M) for the myrosinase inactivation, a previous paper demonstrated that the acidified extract of papaya seed showed a biological activity similar to that of an untreated control extract (*26*). In contrast, glucosinolate metabolism can also occur in the colon as a result of bacterial fermentation (*4*−*6*). Thus, biologically active glucosinolate breakdown products including **1** can be derived from both raw and myrosinase-inhibited papaya seeds.

Inhibition of Superoxide Generation by Extracts from Papaya Seeds and Fruits. We examined the inhibitory effect of the isothiocyanate-containing papaya seed *n*-hexane extract on the TPA-induced superoxide generation because **1** is reported to significantly inhibit this phenomenon (*14*). The superoxide generation was detected by a cytochrome *c* reduction method. As shown in **Figure 3A**, the *n*-hexane extract of the papaya grated seed preparation dose-dependently inhibited the superoxide generation with an IC_{50} value of 10 μg/mL. The seed extract at 25 μg/mL suppressed it by 90%, whereas the pulp extract showed no effect even at 100 μg/mL. The inhibitory activity of the seed is slightly weaker, but comparable to that

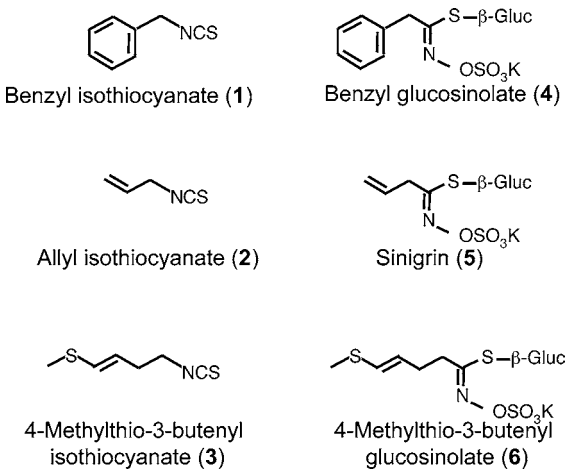

Figure 2. Structures of isothiocyanates (**1**–**3**) and glucosinolates (**4**–**6**) used in this study.

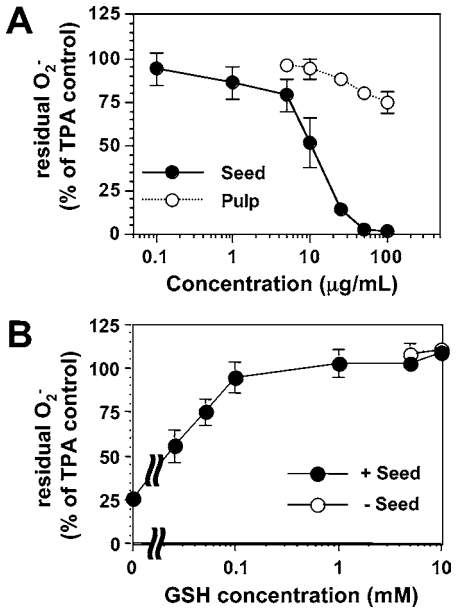

Figure 3. Inhibition of NADPH oxidase-derived superoxide generation in the differentiated HL-60 cells: (**A**) dose-dependent effect of *n*-hexane extract of papaya seeds (●) and pulp (○) on TPA-induced superoxide generation; (**B**) effect of glutathione (GSH) on superoxide inhibition by papaya seed extract. Data were means ± SD ($n = 3$). No error bar means that the standard deviation for the experiments was within 5%.

of **1** as previously reported (approximately 10 μM) (*14*). The electrophilic reactivity is essential to express the inhibitory activity because cotreatment of an electrophile acceptor, glutathione (GSH), with the papaya seed extract counteracted the effect in a dose-dependent manner (**Figure 3B**). These results suggested that electrophilic compounds including **1** play a major role in the inhibition by papaya seeds regarding superoxide generation in differentiated HL-60 cells.

Apoptosis Induction by Papaya Seed Extract in HL-60 Cells. In previous studies, **1** showed a cytotoxic effect in several cultured cell lines (*13*, *15*, *17*, *27*−*33*). Therefore, we monitored the cytotoxic activity of the papaya extracts in the HL-60 cells using the modified MTT assay (*17*). When HL-60 cells were incubated with the papaya seed extract, the viability was inhibited in a concentration-dependent manner up to 100 μg/mL with an IC_{50} value of approximately 20 μg/mL (**Figure 4**). The pulp extract showed no effect even at 100 μg/mL. The cytotoxicity of the seed *n*-hexane extract is slightly weaker than

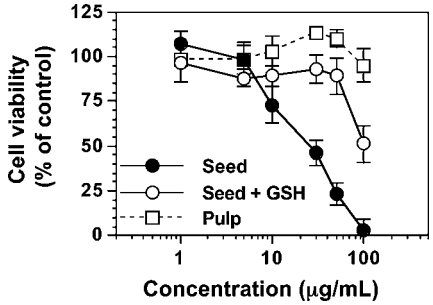

Figure 4. Dose-dependent effect of papaya extract on cell viability of HL-60 cells. Values are mean ± SD ($n = 3$).

that of **1** (approximately 15 μM, data not shown). The electrophilic reactivity is also necessary to express the cytotoxicity because cotreatment of 2.5 mM GSH with the papaya seed extract suppressed it. Next, we explored the cell death mode; the cells were stained with PI and then the cell cycle phase distributions (percent) were measured. The flow cytometric analysis of HL-60 cells exposed to the papaya seed extract for 24 h indicated that 30 and 50 μg/mL concentrations of the extract resulted in the dominant ratio of sub-G1 degraded cells, whereas the ratio of the other cell cycle phases had not significantly changed (**Figure 5**). The ratio of the cells at the sub-G1 region was 62% when the cells were treated with 50 μg/mL of the seed extract, whereas >80% of the cells were killed, detected by the modified MTT assay (**Figure 4**). This difference might be due to necrotic dead cells, based on the previous study showing that **1** induces both apoptosis and necrosis (*13*). Consistent with this, treatment of the cells with 50 μg/mL of the seed extract resulted in significant DNA ladder formation, which is one of the hallmarks of apoptosis, coincidnt with the smear-like pattern of DNA, termed as necrosis (**Figure 6**, lane 3). Conversely, when the cells were cotreated with 2.5 mM GSH, DNA fragmentation was completely inhibited, which is a tendency similar to the cytotoxicity assay. Consistent with our previous report that caspase-3 was activated by BITC (*13*), z-VAD-fmk, a caspase inhibitor, was capable of inhibiting the DNA fragmentation by the seed extract (data not shown). The gathered data indicated that the cell death form induced by the papaya seed extract is apoptosis in dose- and electrophilic reaction-dependent manners.

Compound **4** and myrosinase are reported to be present in the water extract of ground papaya seeds (*19*). Our results show that the extraction of an aqueous grated seed preparation leads to a significant recovery of biological activities and **1** in the *n*-hexane extract. We noted that the differences in the biological activities between the seed and pulp preparations were paralleled by changes in their **1** content, although they contained other types of compounds. A previous paper implied that the aqueous extract of the papaya seeds does not appear to contain enough cyanogenic glucosides to be toxic to nematodes, nor is there any detactable benzyl thiocyanate, benzyl cyanide, and benzonitrile possibly formed by the myrosinase-like hydrolysis reaction of **4** (*26*). Another paper demonstrated that aromatic substances, such as benzaldehyde, benzyl alcohol, 2-phenylethanol, and benzyl isothiocyanate as well as the monoterpene alcohols linalool and 2,6-dimethyloct-7-ene-2,3,6-triol, were detected from the hydrolysis of enzyme-treated papaya fruits (*34*). However, because their distribution in seed and pulp is unclear, their contribution to the cytotoxic effect of the papaya seed extract remains to be elucidated. Anyway, cotreatment of GSH with the papaya seed *n*-hexane extract suppressed each biological activity that **1** exhibited, as previously reported. Taken together, our results indicate that the main principle in the

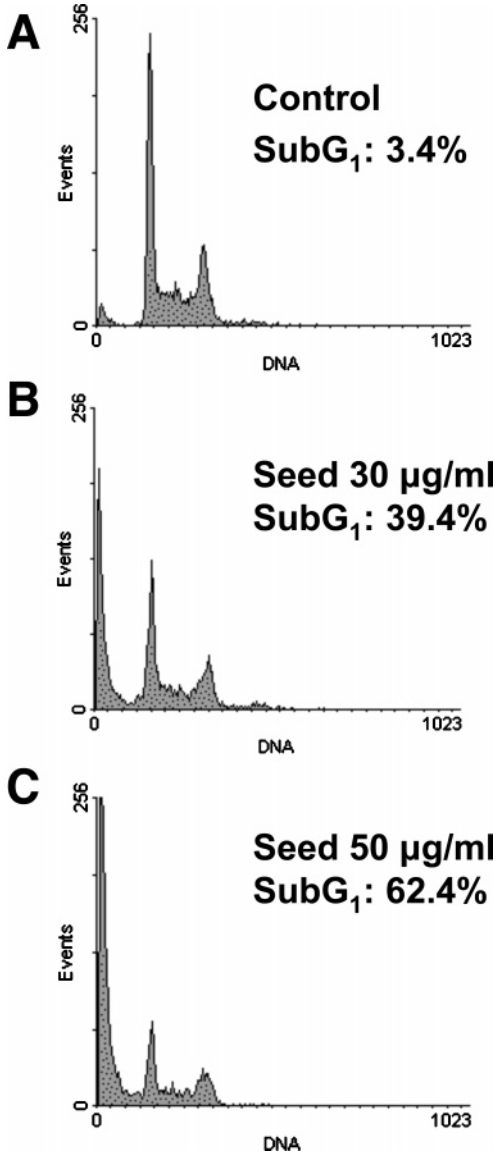

Figure 5. Effect of papaya seed extract on cell cycle distibution in HL-60 cells.

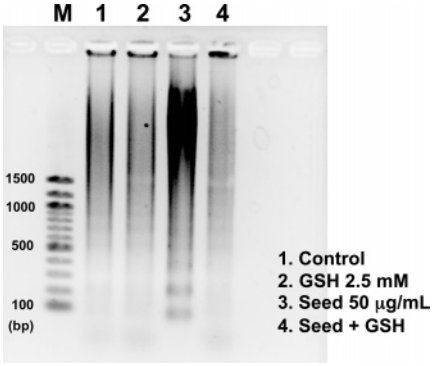

Figure 6. DNA fragmentation in the papaya seed extract-treated HL-60 cells: lane 1, control; lane 2, 2.5 mM GSH; lane 3, 50 μg/mLseed extract; lane 4, 2.5 mM GSH + 50 μg/mL seed extract.

papaya seed preparation we investigated might be an electrophilic compound such as **1**.

The exposure of cells to isothiocyanates has been demonstrated to lead to a rapid and high intracellular accumulation (*35*). Reduced GSH, which is the most abundant thiol-carrying molecule in a cell (1−10 mM in cells), is known to be primarily responsible for the conjugation of isothiocyanates, which results in the formation of dithiocarbamates (R−NH−CS−S−R′). GSH is also the major driving force for isothiocyanate accumulation (*35*, *36*), which is further enhanced by GSH *S*-transferase (GST) (*35*). Intracellular isothiocyanate accumulation is quite rapid, and its levels may reach 100−200-fold over the extracellular concentrations. For example, when murine hepatoma Hepa 1c1c7 cells were treated with 10 μM **1** or 50 μM sulforaphane for 30 min, the total intracellular levels were 1.9 and 5.1 mM, respectively (*36*). The exposure of cells to 50 μM **1** for 30 min consistently depleted GSH by approximately 50% (*12*, *36*). Consistent with this, we observed a significant GSH depletion by 30% to the control in the papaya seed extract (30 μg/mL)-treated HL-60 cells for 30 min (data not shown). The depletion of GSH and other thiol molecules almost exclusively renders the cells more susceptible to oxidative stress and stress-dependent signaling pathways. It is also noteworthy that such a covalent conjugation with GSH is reversible and that the conjugated species can undergo either dissociation or replacement reactions (*37*). Several isothiocyanates, having an electrophilic property, are effective for inhibiting glyceraldehyde-3-phosphate dehydrogenase, a model protein having active thiol groups, whereas the nonelectrophilic *O*-methyl benzylthiocarbamate (Bz−NH−CS−O−CH_3) does not (*14*). This structure−activity relationship has also been found in some biological activities in cultured cells and animals, including the suppression of the NADPH oxidase-dependent superoxide generation (*14*), induction of GST (*11*), and mitochondrial function modification (*16*). Furthermore, the extracellular trapping of **1** by exogenously added GSH inhibits each biological response induced by **1** (*14*), consistent with the present results. Therefore, it is very likely that **1** in the papaya seeds can modify other intracellular thiol molecules including protein sulfhydryls within the cells.

In the present study, papaya seed, but not papaya pulp, is a rich source of biologically active isothiocyanate, especially **1**, and its precursor, glucosinolate, the contents of which are as high as those in *Brassica* vegetables. As mentioned above, compound **1** has been paid less attention because of its rarity in daily foodstuffs such as Brassicaceae vegetables. On the contrary, the present findings provide evidence showing that the papaya seed extract exhibits the same biological effects as does **1** such as superoxide generation inhibition and apoptosis induction. Also, there have been various studies showing the ability of **1** to inhibit chemically induced cancer development or growth of cancer xenografts in vivo (*5*−*7*). The gathered data thus implicated the use of papaya seeds for the prevention and medication of inflammation-related disorders, including cancer. The papaya seed preparation has been used for decades not only in parts of Asia and South America as a vermifugal agent but also used in folk medicine to facilitate good menstrual flow or abortifacient effects, in both of which **1** might be involved. In the Ayurverdic system of traditional medicine, 0.5−1 g of powdered papaya seeds is prescribed for medicinal use (*38*), although the concentration of **1** might be dependent on the environment/origin and maturity of the papaya fruits from which the seeds were harvested (*39*, *40*). Therefore, the exact amount of **1** delivered to human systems via papaya seed consumption could vary. The appraisal of the safety of papaya seed preparations is necessary to protect the populace from the potential hazards of its use. Because the papaya seeds are not consumable in daily life compared with cruciferous vegetables, further investigation of papaya seed processing or effective extraction of glucosinolate is necessary and will contribute to practical applications to humans.

ABBREVIATIONS USED

GST, glutathione *S*-transferase; GSH glutathione, TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

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