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# Interleukin-8 stimulating activity of low molecular weight β-glucan depolymerized by γ-irradiation

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ABSTRACT

Since many studies reveal that the biological properties of various biopolymers depend on their molecular weight. Therefore, it was considered of importance to investigate how molecular weight affected on the interleukin-8 (IL-8) stimulating activity of the β-glucan from *Ophiocordyceps dipterigena* BCC2073. γ-Irradiation of the glucan with various doses (0–100 kGy) was chosen as a clean method to produce different low molecular weight samples. The result showed that average molecular weight (MW) of irradiated samples significantly decreased as the irradiation dose increased whereas the functional groups of before and after irradiated glucans detected by FTIR and $^{13}$C-NMR were identical. However, difference in intensity of an absorption peak at 270 nm was found in the UV/vis spectra. The biological properties such as cytotoxicity, proliferation and IL-8 secretion of normal human dermal fibroblast contacted with various MW glucans were also tested. It was found that the glucan with MW of approximately 5 kDa exhibited the highest ability to induce IL-8 production. Apart from the MW effect, chain conformation seems to be involved. Thus, differences in solution conformation of various MW glucans via Congo red analysis were evaluated.



## 1. Introduction

Many microorganisms produce polysaccharides which are essential biomacromolecules in their activities and play an important role in molecular recognition in immune system. β-Glucan is a well-known biological response modifier widely distributed in nature (Bohn & BeMiller, 1995). A variety of β-glucan has been isolated from various sources, for examples, fungi, yeast, bacteria, oats and barley. Recent reports have highlighted a significant role of β-glucan in the treatment of cancer and infectious diseases in both modern medicine and traditional oriental therapies (Chen & Seviour, 2007; Mantovani et al., 2008). They also play an important role as dietary fibers because they reduce the plasma cholesterol level (Kalra & Jood, 2000) and enhance the hematopoietic response (Hofer & Pospisil, 1997). Because the high molecular weight of glucan caused some problems such as high viscosity and low permeability into cell, several degradation methods have been reported. The depolymerization methods by using chemical or enzymatic hydrolysis have been used to improve the functional properties of biopolymers (Jeon & Kim, 2002; Li et al., 2010). Although these methods are effective in decreasing the molecular weight, they do have certain disadvantages such as a high cost, low yield, long processing time, and disposal of wastes. γ-Irradiation is an ionic, no-heat process that continues to receive attention as a preservation and functional modification method in polymer research and application. In comparison with other physical modification methods, such as microwave, sonic wave, UV, ultrahigh hydrostatic pressure and hydrothermal treatment, γ-irradiation is rapid, convenient and the most efficient polymer degradation method (Wasikiewicz, Yoshii, Nagasawa, Wach, & Mitomo, 2005). In addition, this technique is more environmentally friendly than the conventional methods. Recently, β-glucan from black yeast depolymerized by gamma irradiation has been reported (Byun et al., 2008; Sung et al., 2009). Apart from molecular weight, chain conformation of the polysaccharides can significantly affect their biological activities (Leung, Liu, Koon, & Fung, 2006). However, the contradiction of β-glucan chain conformers in immuno-enhancing activity has been reported (Leung et al., 2006).

Regarding wound healing process, it proceeds in 3 overlapping phases: inflammation, granulation tissue formation, and matrix and remodeling phase. This sequential process is believed requiring the interaction of cells in the dermis and epidermis as well as the activities of chemical mediators released from inflammatory cells, fibroblasts and keratinocytes (Tsuboi & Rifkin, 1990). Cytokines

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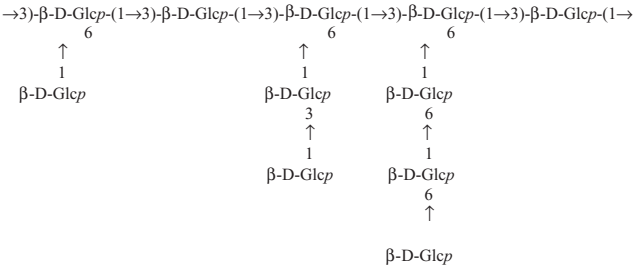


**Fig. 1.** Chemical structure of the fungal glucan from *Ophiocordyceps dipterigen*a BCC 2073.

are known as biological response modifiers that modulates inflammation, immunity and hematopoiesis. Interleukin-8 (IL-8) is also an important cytokine implicated in inflammation and postnatal wound healing. It has been shown to stimulate chemotaxis of neutrophils and keratinocytes, as well as stimulating neovascularization (Liechty, Crombleholme, Cass, Martin, & Adzick, 1998). In our previous work, it has been reported that the exopolysaccharide secreted from Entomopathogenic fungus, *Ophiocordyceps dipterigena* BCC 2073, is biocompatible and strong inducer of IL-8 which is a cytokine responsible for enhancing wound healing process (Kocharin, Rachathewee, Sanglier, & Prathumpai, 2010; Madla, Methacanon, Prasitsil, & Kirtikara, 2005). This fungi-derived polymer was found to be mainly composed of (1 → 3)-β-D-glucan substituted with side chains of (1 → 6)-β-D-glucopyranosyl units (Methacanon, Madla, Kirtikara, & Prasitsil, 2005) as shown in Fig. 1. It was therefore interesting to elucidate the relation among molecular weight, chain conformation and IL-8 stimulating activity of the studied molecular weight β-glucans.

## 2. Experimental

### 2.1. Microorganism and culture condition

Entomopathogenic fungus *Ophiocordyceps dipterigena* BCC 2073 was kindly provided by Dr. Nigel L. Hywel-Jones, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Thailand and has been deposited in BIOTEC Culture Collection.

#### 2.1.1. Inoculum

The fungus was initially grown on potato dextrose agar (PDA) at 25 °C for 5–7 days. An agar block (1 $cm^3$) containing mycelia was cut into small pieces and then transferred to 25 ml potato dextrose broth (PDB) and incubated for 5–7 days at 25 °C in a rotary shaker (200 rpm) (New Brunswick, NJ, USA).

#### 2.1.2. Fermentation condition

The medium in 5 l fermenter (Marubishi Co., Ltd., Pathumthani, Thailand) with a working volume of 4 l contained 60 g/l glucose, 14 g/l malt extract, 0.5 g/l $KH_2PO_4$, 0.2 g/l $K_2HPO_4$, 0.2 g/l $MgSO_4{\cdot}7H_2O$, 0.14 g/l $MnSO_4{\cdot}H_2O$, and 1 ml/l vitamin solution (Blackmores, NSW, Australia). Vitamin complex consisted of 75 mg of vitamin B1 (thiamine hydrochloride), 10 mg of Vitamin B2 (riboflavin), 50 mg of nicotinamide, 25 mg of calcium pantothenate, 10 mg of vitamin B6 (pyridoxine hydrochloride), 25 mg of vitamin B12 (cyanocobalamin), 15 mg of biotin, 500 mg of vitamin C (derived from ascorbic acid 260 mg and calcium ascorbate 290.5 mg), 10 mg of choline bitartrate, 10 mg of inositol, 10 mg of zinc amino acid chelate (zinc 2 mg), 175 mg of calcium phosphate, and 75 mg of magnesium phosphate. The trace element solution contained (per l) 14.3 g of $ZnSO_4{\cdot}H_2O$, 2.5 g of $CuSO_4{\cdot}5H_2O$, 0.5 g of $NiCl_2{\cdot}6H_2O$ and 13.8 g of $FeSO_4{\cdot}H_2O$. The culture was agitated at 300 rpm, aerated at 1 vvm, temperature 25 °C and pH was not controlled.

### 2.2. Isolation and purification

Extracellular materials were isolated and purified as reported in Madla et al. (2005). Briefly, after fermentation, distilled water was added to the crude broth and any insoluble materials were removed by centrifugation. Then, polymer was separated by precipitation of supernatant in ethanol, dialysis against distilled water for 24 h and lyophilization.

### 2.3. Total carbohydrates and proteins contents

Total carbohydrates and protein contents of the sample were determined by phenol–sulfuric acid assay using D-glucose (Chaplin, 1986) and Lowry protein assay using bovine serum albumin (BSA) as a standard (Stoscheck, 1990), respectively.

### 2.4. Gamma irradiation

γ-Irradiation of the glucan solution was carried out using a cobalt-60 irradiator (Gammacell 220 Excel, MDS Nordion). The doses applied in this work were 0, 5, 10, 25, 50, and 100 kGy at room temperature. The source strength was approximately 11.557 kCi (427.6 TBq) with a dose rate of 9.4 kGy/h on March 2009. Dosimetry was carried out using 10 mm × 30 mm × 3 mm of Harwell Red Perspex 4034 (Harwell Dosimeters Ltd., UK) calibrated against reference standard dosimeter (Fricke dosimeter) that was traceable to international standard set by the Office of Atoms for Peace, Bangkok, Thailand.

### 2.5. Instrumental methods

#### 2.5.1. Gel permeation chromatography

The molecular weight of samples was estimated by gel permeation chromatography (GPC) using dextran (MW 4400–401,000) as a standard. Analysis was performed on Ultrahydrogel linear column (7.8 i.d. × 300 mm, Waters, USA) connected in line with a Waters GPC apparatus. Samples (2 mg/ml) were eluted with 0.05 M sodium bicarbonate buffer (pH 11) with a flow rate of 0.6 ml/min at 25 °C and monitored by RI detector.

#### 2.5.2. FT-IR

FT-IR spectra were collected on a FT-IR/FT-Raman (Perkin Elmer System 2000) with TGS (triglycine sulphate) detector as an average of 16 scans. The spectra were recorded from a KBr disc containing 1% finely ground samples in the transmittance mode from 4000 to 600 $cm^{-1}$ at a resolution of 4 $cm^{-1}$.

#### 2.5.3. $^{13}$C-NMR

The $^{13}$C-NMR spectra were recorded at 298 K in 0.1 M NaOD (50 mg/ml) using an AVANCE 300 MHz Digital NMR Spectrometer (Bruker Biospin, AV-500). The spectra were recorded at a frequency of 125 MHz using parameter as follows: 20,000 numbers of scan, relaxation delay of 1 s, spin rate of 25 Hz and spectral size 32K with 32K time domain size.

#### 2.5.4. UV/vis

UV/vis spectra of irradiated samples were recorded between 200 and 500 nm using an UV/vis spectrophotometer (JASCO V-530, Japan). The samples were completely dissolved in 0.1 M NaOH before scanning.

### 2.6. Cytotoxicity and IL-8 production

Normal human dermal fibroblasts were seeded into 96-well microplates at a concentration of $3 \times 10^3$ cells/well in Dubelco's modified Eagle media (DMEM) supplemented with 10% fetal bovine serum and incubated at 37 °C. After 48 h of incubation, the media were replaced with fresh media containing 50 μg/ml of sample irradiated at various doses and re-incubated for additional 48 h. Media were then collected and the level of IL-8 was determined using an IL-8 ELISA test kit (R&D Systems Europe). Subsequently, the fresh media and 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were added into the wells and the whole microplates were again incubated at 37 °C for 4 h. The collected purple formazan products were ultimately dissolved in dimethyl sulfoxide (DMSO) and glycine buffer for the measurement of optical density values using a Microplate Reader at 570 nm. Finally, the obtained values were converted to percentage of cell viability. Cells without glucans acting as a control group were performed in the same manner as described above.

### 2.7. Congo red analysis

A caustic soda dilution series was prepared (0.025–0.5 M). Each sample (approx. 10 mg) was dissolved in 1000 μl of the caustic soda solution, 1 ml Congo red solution (30 mg/l) was then added and the resulting solution was placed in a cell (pathlength 1 cm). The wavelength at which maximum absorption occurs was determined by an UV/vis spectrophotometer (JASCO V-530, Japan). An analogous series without the sample was performed as a reference.

## 3. Results and discussion

### 3.1. Changes in molecular weight

In this study, γ-irradiation was used as an effective method due to its advantages including the ability to promote changes reproducibly and quantitatively, without the introduction of chemical reagents, to produce various low molecular weight β-glucans. Initially, total carbohydrates and protein contents of glucan sample before irradiation were calculated as 98.18% and 2.82%, respectively, which indicated the presence of polysaccharide as a majority. Subsequently, samples were irradiated with γ-ray at various doses (i.e. 5, 10, 25, 50, and 100 kGy) for further studies. It has been known that effect of chain scission can be followed by decreasing in molecular weight of polysaccharides. Average molecular weights at peak ($M_p$) of the glucans exposed to γ-radiation at various doses are shown in GPC chromatograms (Fig. 2). As expected, the results showed degradation of the polysaccharide chain in different degrees under the studied irradiation conditions. Clearly, the molecular weight decreased significantly with increasing dose. The average molecular weight and polydispersity of non-irradiated sample were found to be 590 kDa and 2.1, respectively whereas those of irradiated samples ranged from 5.2 to 120 kDa, and the polydispersity were between 1.4 and 2.4. It is worth noting that the molecular weight decreases rapidly at first, subsequently levels off to much slower rate at dosage more than 5 kGy, and after that the molecular weight remains almost unchanged at the oligomer level. The degradation result found in this study are well supported by reports of depolymerization by radiolysis of many polysaccharides such as starch (Wu, Shu, Wang, & Xia, 2002), chitosan (Hai, Diep, Nagasawa, Yoshii, & Kume, 2003), alginate (Nagasawa, Mitomo, Yoshii, & Kume, 2000), and konjac glucomannan (Xu, Sun, Yang, Ding, & Pang, 2007). A decrease in the average molecular weight of the irradiated samples after exposed the γ-radiation results from the breakage of the glycosidic bond of polysaccharide (Byun et al., 2008) which is attributed to free radical reactions following with chain scission (Xu et al., 2007). In general, the extent of degradation of the polysaccharides is not linearly proportional with the dosage of ionizing radiation. In the low-dose range, the degradation of the polysaccharide is greater than that under higher dosage (Choi et al., 2009). In addition, it has been reported that degradation rate increases with decreasing of polymer concentration, which is mainly caused by the enhanced hydroxyl free radical (•OH) mobility in dilute solution due to reduced viscosity. Moreover, in dilute solution the distances between two radicals located on neighboring polymer chains become larger, resulting in less cross-linking reaction possibility (Wasikiewicz et al., 2005).

### 3.2. FT-IR

Since FT-IR spectroscopy has been shown to be a useful tool in monitoring structural changes in biopolymers, the glucan samples before and after irradiation were subjected to FT-IR analysis. The FT-IR spectra of the glucan irradiated with γ-radiation were recorded as shown in Fig. 3. It was found that there was very similar in FT-IR spectra of all samples irradiated at 0–100 kGy, indicating that there are no any significant changes in the core structure of glucan even though they possessed different molecular weights. This is in accordance with the results obtained by Byun et al. (2008). A strong band at 3350 $cm^{-1}$ was assigned to the hydroxyl stretching vibration of the polysaccharide. The bands in the region of 2926 and 1745 $cm^{-1}$ were due to C–H stretching vibration and C=O stretching (ester), respectively. The unusual absorption at 1745 $cm^{-1}$ in these glucans possibly indicates the presence of lipid ester which also has been found in extracellular matrix secreted by *Botrytis cinerea* (fungal pathogen of plant) (Doss, 1999) and glucans from fruit bodies of mushrooms *Pleurotus ostreatus* and *Pleurotus eryngii* (Synytsya, Míčková, Synytsya, Jablonský, & S, 2009). Another characteristic peak occurred at 1642 $cm^{-1}$ is presumably a feature of tightly bound water in the biopolymers. Bands at 1419 and 1364 $cm^{-1}$ were attributed to the $CH_2$ bending and twisting, respectively. The peaks around 1022, 1079 and 1151 $cm^{-1}$ typified the characteristics of the coupling of C–O and C–C stretching modes, C–OH stretching, and C–O–C asymmetry stretching (glycosidic bonds), respectively (Copikova, Synysya, Cerna, Kaasova, & Novotna, 2001). Generally, band in the region of 850–950 $cm^{-1}$, corresponding to the skeleton mode of the anomeric configuration (α or β). An expected absorption at approximately 890 $cm^{-1}$ which is a characteristic band of a β-configuration was not differentiated whereas the presence of α-glucan was shown by the prevailing band at approximately 850 $cm^{-1}$ (Rout, Mondal, Chakraborty, Pramanik, & Islam, 2005).

### 3.3. $^{13}C$-NMR

$^{13}C$-NMR spectrum of the glucan before irradiation is shown in Fig. 4. It is worth noting that all NMR spectra of the glucans after irradiation were identical to that before irradiation, as well as the results obtained from FT-IR. However, in the NMR, the β-configuration of the D-glucopyranosyl residues was clearly evidenced by the presence of anomeric peak (C-1) at approximately 103 ppm. Spitting of this peak was attributed to presence of both β-(1 → 3) and β-(1 → 6). In addition, the data reveal peak corresponding to the α-configuration of the anomeric carbon which would resonate at 100.8 ppm. An approximate β- and α-glucan ratio could be calculated as about 2:1. Another characteristic signal of β-glucan was at approximately 81 ppm assigned to C-3. The downfield shift of C-3 by 4 ppm was due to α-effect of glycosylation. Generally, the glycosylation of a carbon atom creates a downfield shift of 4–10 ppm of α-carbon and an upfield shift of 1 ppm for β-carbon. The effects of glycosylation depend on the configuration of the anomeric centre of the glycosylating sugar residue

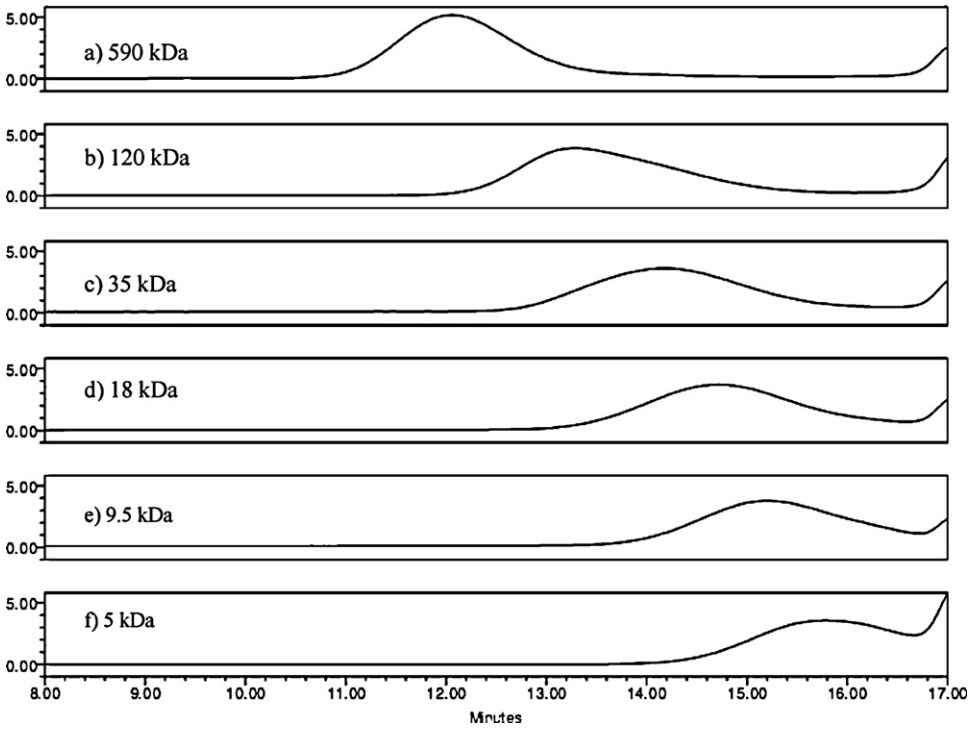


**Fig. 2.** GPC chromatograms of glucan irradiated at 0 kGy (a), 5 kGy (b), 10 kGy (c), 25 kGy (d), 50 kGy (e), and 100 kGy (f).

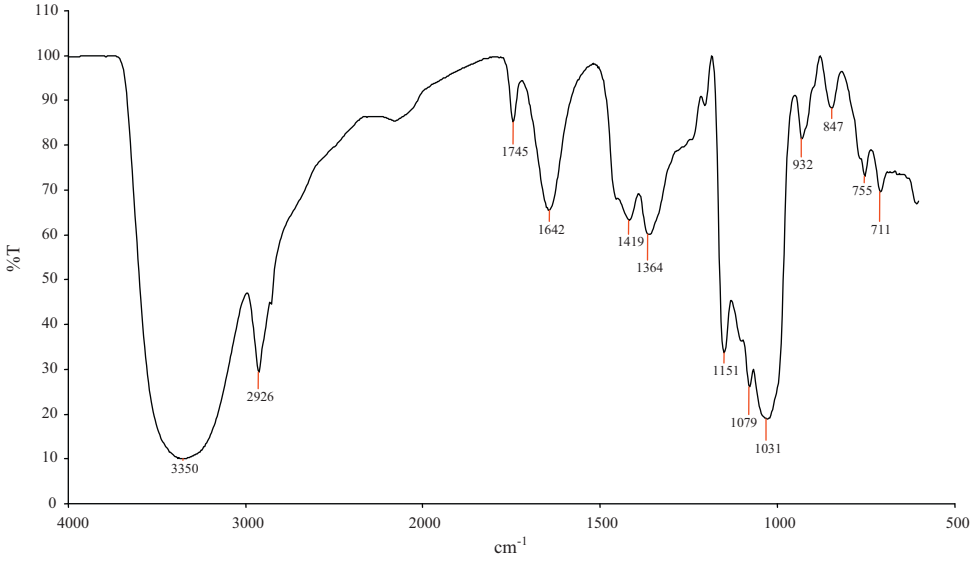


**Fig. 3.** FT-IR spectrum of the irradiated glucan. The similar spectra were obtained for glucans with molecular weight 5–590 kDa.

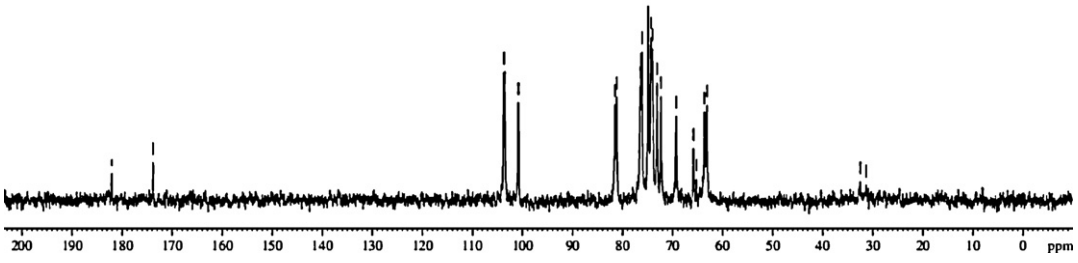


**Fig. 4.** $^{13}$C-NMR spectrum of glucan. The similar spectra were obtained for glucans with molecular weight 5–590 kDa.

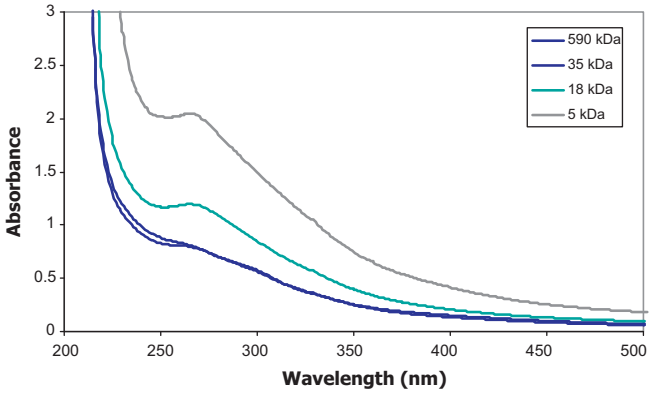


**Fig. 5.** UV/vis spectra of glucans with different molecular weights.

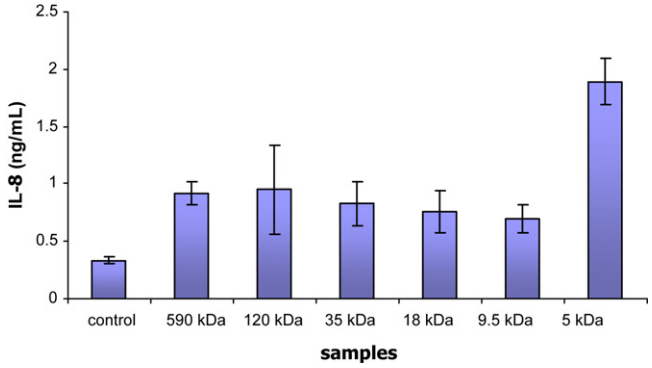


**Fig. 6.** IL-8 production of normal human dermal fibroblasts exposed to glucans with different molecular weights.

(glycone) and on the relative absolute configuration of the glycone and aglycone. When at least one of the carbons of the aglycone, adjacent to the glycosylated carbon, bears an equatorial proton, this dependence is well accounted for by a strengthening or weakening of the spatial interaction between the protons of the glycone and aglycone, caused by a change in the conformation around the glycosidic linkage (Shashkov, Lipkind, Knirel, & Kochetkov, 1988). Other signals around 76, 74, 69, and 63 ppm attributed to C-5, C-2, C-4, and C-6, respectively, corresponding with many literatures (Chakraborty, Mondal, Rout, & Islam, 2006; Schmid et al., 2001; Wang & Zhang, 2009). Chemical shifts corresponded to carbonyl (ester) and alkyl groups were noticeable at 170–180 ppm and 30 ppm, which was well correlated with the FTIR result.

### 3.4. UV/vis

Degradation process was also monitored by UV/vis spectrophotometry. As shown in Fig. 5, the UV/vis spectra of irradiated glucans with various molecular weights showed the presence of absorption band and its intensity at 270 nm. The increase in the absorbance with decreasing of molecular weight (increasing radiation dose) could be assigned to formation of carbonyl group or double bonds of glucan formed after main chain scission by irradiation (Xu et al., 2007). Although, the slightly shifted absorbance maximum was observed in this study, this result is still consistent with many previous works. For instance, irradiation of alginate (Nagasawa et al., 2000), konjac glucomannan (Xu et al., 2007), and hyaluronic acid (Kim et al., 2008) showed the increase of UV absorbance at 265 nm while UV peaks at 280 nm and 250 nm could be due to the formation of carboxyl group in fucoidan and carbonyl group in laminarin, respectively (Choi et al., 2009). Irradiation of chitosan also showed the increase of UV absorbance at 290 and 247 nm (Ulanski & Rosiak, 1992).

### 3.5. Cytotoxicity and IL-8 production

The viability of normal human dermal fibroblasts after exposure to the β-glucan with various molecular weights for 48 h was studied. It was found that all samples showed to be non-toxic to human cells since % cell viability exceeded 70% (data not shown). Unfortunately, none of samples could promote the proliferation of the cells since the % cell viability did not exceed 100%. For IL-8 production of β-glucan (Fig. 6), the result indicated that the molecular weight of β-glucan significantly affected the IL-8 production of the dermal fibroblasts. The sample irradiated at 100 kGy possessing the lowest molecular weight of 5.2 kDa apparently stimulated the fibroblasts to produce the highest IL-8 content (2.38 ng/ml). This result is in good agreement with the study of Sung et al. which reported that immune response of β-glucan was more effective at low molecular weight ranging from 1 to 30 kDa than at high molecular weight (Sung et al., 2009). On the other hand, some works also reported that (1 → 3)-β-glucans with medicinal properties are strongly dependent on high molecular weight, ranging from 500 to 2000 kDa (Zhang, Cui, Cheung, & Wang, 2007). Meanwhile, the glucan samples irradiated between 0 and 50 kGy with molecular weight ranging from 120 to 9.2 kDa stimulated the fibroblasts to produce IL-8 about 0.73–1.28 ng/ml. Nevertheless, it is notable that in this studied molecular weight range the IL-8 production is not directly proportional to molecular weight. The IL-8 content gradually raised to a maximum value at molecular weight of 35 kDa (at 10 kGy) and then dropped to the lowest value at molecular weight of 9.5 kDa (at 50 kGy). Since it has been reported that helical conformation is regarded as an important structural feature by playing a significant role in the biological recognition within cells (Zhang et al., 2007), the differences in stimulation efficiency seen within the irradiated glucan samples were considered to be not only an effect of molecular weight but also an effect of different chain conformation of the irradiated glucans.

### 3.6. Solution chain conformation

In order to elucidate the influence of the chain conformation on the IL-8 production, the different molecular weight glucans were subjected to Congo red analysis. The formation of the Congo red–glucan complex and the resulting shift in the maximum absorption wavelength ($\lambda_{max}$) of Congo red is a rapid method for detecting helical structures. Fig. 7 shows the shift of $\lambda_{max}$ at different concentrations of NaOH in the presence of the glucans irradiated at 10, 25, and 50 kGy. At low NaOH concentrations ($\leq$0.05 M), the $\lambda_{max}$ of the Congo red–glucan complex shifted to a longer wavelength (from 485 to 490 nm), reaching the longest wavelength at 0.1 M NaOH ($\lambda_{max}$ 500–505 nm). At higher concentrations, the $\lambda_{max}$ dropped rapidly and leveled off at approximately 480 nm which is almost the same value as that of the control solution (Congo red dye). Change in the $\lambda_{max}$ of the Congo red in the presence of these irradiated glucans, which is consistent with an order-disorder transition, indicated the existence of helix conformation. This finding is consistent with many previous works that demonstrated (1 → 3)-β-glucans with helix conformation forming complexes with Congo red in dilute alkaline solutions (Kath, Lange, & Kulicke, 1999, Zhang et al., 2007). An increase in pH will also shift the conformation from helix conformation to random coil structure, which may be attributed to the breakage of hydrogen bonds (William et al., 1991). On the other hand, for untreated glucan (590 kDa) and glucans with molecular weight of 120 and 5 kDa, no significant shift in $\lambda_{max}$ was observed, indicating the absence of helical structure. Surprisingly, the helix conformation was not found in the untreated glucan, which could possibly be attributed to

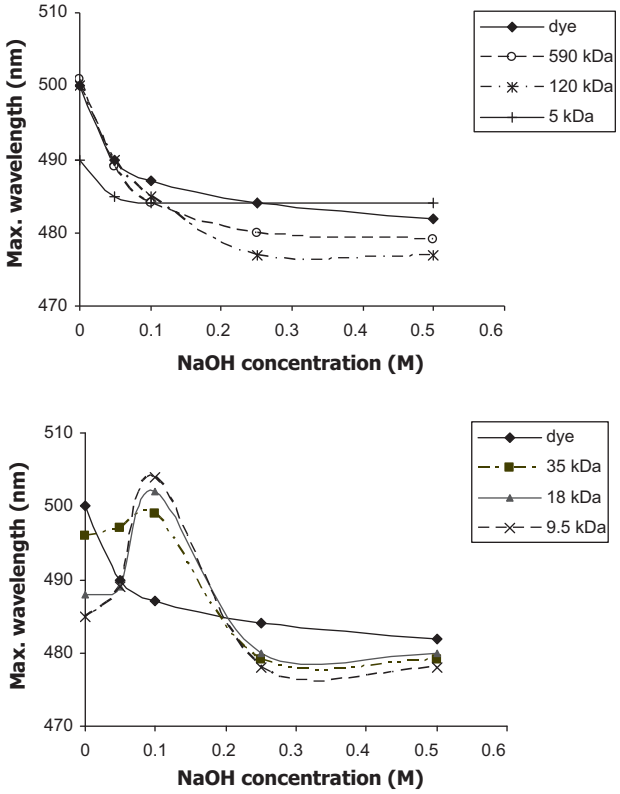


**Fig. 7.** Absorption maxima of Congo red solutions containing various molecular weight glucans.

the effect of branching degree since the substituents are bulky and disrupt the helix conformation of the chain. Both positive and negative correlations between branching degree and biological activity have been reported (Leung et al., 2006). For this work, the result showed that highly branched chains represent obstacles for molecular formation as helix chain, as in the case of the untreated glucan (590 kDa) and the glucan with molecular weight of 120 kDa. When the irradiation dose was increased, chain scission seems to occur mostly at branched chains. As the consequence, less branching structure resulted in easier helix formation of glucan molecules, as well as for glucans with molecular weight ranging from 35 to 9.5 kDa. The results suggested that the glucan with helix conformation (due to its low degree of branching) is less active in inducing IL-8 production. For glucan with molecular weight of 5 kDa, the whole polymer chains seemed to be broken down into very short chains possessing random coil conformation. Moreover, this very low molecular weight glucan with random coil structure showed the pronounced effect, which presumably smaller molecules have better chance in binding to more receptors.

In conclusion, chain conformation result correlated well with the glucans ability to induce IL-8 production. Two possible mechanisms are therefore proposed here. Firstly, the IL-8 stimulating activity of high molecular weight glucans is attributed to chain conformation that is either random coil or less helical structure. Secondly, for low molecular weight glucan, the activity is enhanced by suitable molecular size (ca. 5 kDa).

## 4. Conclusions

In this study, γ-irradiation was shown to be an effective method to produce various low-molecular weight β-glucan without noticeable changes in functional groups of the glucan as detected by FTIR and $^{13}$C-NMR. The biological result also indicated that it has no significant cytoxicity against human fibroblast cells. In addition, the results implied that fibroblasts adhering to β-glucan samples could secrete IL-8 and other cytokines, which in turn could induce angiogenesis, fibrosis and epithelialization. It is also worth noting that the efficacy of β-glucan sample as IL-8 inducer tends to be associated with its molecular weight. In addition, the efficiency in IL-8 inducer of the irradiated glucans was dependent on chain conformation. Either random coil structure or very low molecular weight glucans demonstrated high activity in IL-8 production.

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