Enhanced Expression of Dermal Aquaporins by Polysaccharide from *Camellia sinensis*

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**ABSTRACT**

The green tea polysaccharide was extracted from leaves of *Camellia sinensis* and hydrolyzed with enzyme. Skin moisturization effect of green tea polysaccharide was studied with 2D-PAGE analysis of epidermal hydration factors. Among up-regulated proteins extracted from Hacat cell treated with Green tea polysaccharide on 2DE gel, 5 aquaporins were observed. Among them the aquaporin 5 and 8 were increased 3.4 and 5.3 times respectively. The green tea polysaccharide was found to be effective for skin moisturization by enhanced expression of aquaporins.

**Keywords:** green tea, polysaccharide, moisturization, aquaporin

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Introduction

The epidermis is the outmost part of the skin and it is made up of five layers. The outmost layer of those skin layers is stratum corneum and acts as protective skin barriers. Before the 70’s in last century, the stratum corneum was regarded as bio-inert tissue, seemed to be a thin plastic film that protects the lower layers of the skin. In the past a few years, scientists have found that the stratum corneum has biological and chemical activity that is quite complicated. The stratum corneum has a "brick and mortar" structure in which the "bricks" are corneocytes consisted of protein complexes. Corneocytes are composed of small threads of keratin in an organized matrix. The keratin contains a lot of water among the fibers. The stratum corneum consists of around 12~16 layers of corneocytes and the thickness of corneocyte is average 1μm.

For a long time, scientists believed that the cells are offered with their own channels for water transportation. Water can pass easily and rapidly through those channels. With several years of efforts and studies, in 1992, scientists who made breakthrough eventually discovered water channels in the cell membrane, and then named them aquaporins. [1-5]

The epidermis is the most top layer of skin that does not contain blood vessels. Besides, it does not like most of the other organs of the body which absorb nutrients from the blood, but epidermis through the water circulating to nourish itself. Water not only supplies inorganic salts and other nutrients, but also moisturizes and smooth the skin. 13 different aquaporins (AQP0 to AQP12) have been identified in the human body, but until recently it has been determined that AQP 3 is existed in the epidermis and very effective. AQP3 regulating water transportation plays a key role in influencing skin moisture, skin elasticity and skin barrier function [6-8].

This study was carried out to evaluate the moisturization efficacy of polysaccharide from green tea, Camellia sinensis. Expressions of epidermal aquaporins analyzed to evaluate the skin hydration efficacies by green tea polysaccharides.

Experimental

Green tea polysaccharide was extracted from leaves of Camellia sinensis. Prior to extraction, caffeine and catechins were removed. Polysaccharides extracted were hydrolyzed with viscozyme L. Medium chain polysaccharides were collected and dried. Hacat cell was seeding and after 6 hours cultivation, it was treated with polysaccharide at concentration of 1000 ppm. After 24 hours treatments, cells were harvested. 10^7 cells washed with PBS were isolated by centrifugation. Proteins were extracted by the lysis of cells. The lysis buffer solution was prepared with 7M urea, 2M Thiourea, 4% CHAPS, 1% DTT, 2% Carrier ampholyte, 40 μL/mL PIC, 1 μg/mL Benzonase, 0.002% BPB. Cells in lysis buffer were sonicated for 1 min and maintained for 30 min at the room temperature. The solution was centrifuged at 4000 x g for 5 min. The supernatant was collected and preserved at -20°C. The amount
of total protein extracted was measured with modified Bradford method. Bovine γ-globulin was used as standard protein. Immobiline Dry strips (13cm, pl 3-10 NL, Amersham Biosciences, Sweden) were used with IPGphore fixed length strip holder. The strip was rehydrated with 250 μl of the rehydration solution for 12 hours and 100 μg of the sample proteome was injected simultaneously. The rehydration solution was prepared with 7M urea, 2M Thiourea, 2% CHAPS, 1% DTT, 2% Carrier ampholyte, 10% glycerol, 0.002% BPB. Isoelectric focusing was done in IPGphore (Amersham Biosciences, Sweden). After 12 h of rehydration, stepwise focusing was made, 1 h at 500 V, 1 h at 2000 V, then increased to 8000 V and maintained until no current change was observed. Before the SDS electrophoresis, focused strips were equilibrated in buffer solutions. After the focusing, the strip was immersed in 10 ml of the equilibrium buffer solution (7M urea, 2M Thiourea, 2% SDS, 50mM Tris-HCl, 30% glycerol) with 1% DTT and maintained under the mild shaking for 15 min. Then it was immersed again in the equilibrium buffer solution containing 2.5% iodoacetamide and maintained with mild shaking for 15 min. 10.5% acrylamide homogenous gels (T-13%, C-2.5%, 18x24 cm) were made in 1.5 mm thickness. For stacking, 0.5% low melting agarose was used. The IPG strip was located on the top of the SDS gel with the size marker and the stacking gel was poured. For the stacking, 10 mA was applied for 25 min. For the electrophoresis 35 mA/gel was applied until the bromophenol blue marker striped out. The gel was washed for 1 h with dH2O. It was fixed for 1 h in the fixing solution prepared with 50% methanol and 5% acetic acid. After the repeated washing for 1.5 h in dH2O, the gel was maintained for 5 min in 0.02% sodium thiosulfate solution and then washed twice for 10 min. The staining was made in the refrigerated 0.1% silver nitrate solution for 30 min. After the duplicate washing for a minute, the gel was maintained for 10 min in the developing solution composed of 2% sodium carbonate, 0.014% formaldehyde, and sodium thiosulfate. Once protein spots were recognized, 1% acetic acid was added and maintained 10 min for the fixation. The stained gel was scanned and protein spot images were analyzed with 2D Elite (Amersham Biosciences, Sweden) image analysis software.

Results and Discussion

Composition of green tea polysaccharide

Polysaccharide extracted and enzyme hydrolyzed was analyzed to evaluate its composition. Analysis was made with tandem mass spectrometer(API-150). The polysaccharide used in this study was composed of various saccharides, monosaccharide, disaccharide, oligosaccharide, and polysaccharide as shown in Fig 1.

The quantitative composition of various saccharides were also analyzed according to their m/z value. As shown in Fig 2, oligosaccharide was the most abundant components in the green tea polysaccharide used for studies.
Fig. 1. ESI-MS analysis of green tea polysaccharide. Various saccharides were analyzed according to their m/z values.

Fig. 2. Composition of green tea polysaccharide. ESI-MS analysis showed oligosaccharides were most abundant saccharides.
Proteome analysis for expression of aquaporins

Proteome technique provides an overview of various aquaporins related to skin moisturization. So it can be an efficient tool to find out complicated skin hydration caused by expression of aquaporins. The protein profiling was performed as comparison to expressed protein with and without green tea polysaccharide. Protein spot images in the 24x24 cm gels are shown. Fig. 3. is protein spots from the cell not treated with green tea polysaccharide. Fig. 4, on the other hand, is proteins spots with green tea polysaccharide.

Fig. 3. Proteins spots of control 2DE gel.

Fig. 4: Image of proteins extracted from Hacat cell treated with Green tea polysaccharide on 2DE gel
In the control gel, 1050 spots were identified. And in the gel with green tea polysaccharide, 1376 spots were identified. When the comparison between with and without green tea polysaccharide, 633 spots were matched. Among them 203 spots were down-regulated and 430 spots were up-regulated. Among those up-regulated proteins, we found 5 aquaporins as shown in Fig. 5.

![Fig. 5: Three dimensional images of aquaporins expressed by green tea polysaccharide.](image)

**Table. 1. Summary of increased expression of aquaporins by green tea polysaccharide.**

<table>
<thead>
<tr>
<th>2DE spot proteins</th>
<th>Expression Increased (% of up-regulation compared to the control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquaporins</td>
<td></td>
</tr>
<tr>
<td>AQP-3</td>
<td>161.9</td>
</tr>
<tr>
<td>AQP-4</td>
<td>158.5</td>
</tr>
<tr>
<td>AQP-5</td>
<td>343.0</td>
</tr>
<tr>
<td>AQP-8</td>
<td>534.1</td>
</tr>
<tr>
<td>AQP-9</td>
<td>138.6</td>
</tr>
</tbody>
</table>

Aqaporins are related to epidermal hydration. The enhanced expression of 5 aquaporins represents the green tea polysaccharide is efficient to epidermal skin moisturization. Among various aquaporins, AQP5 was the most much expressed by green tea polysaccharide as shown in Table 1. Up-regulated expression of other aquaporins was also believed to increase epidermal hydration.

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References


