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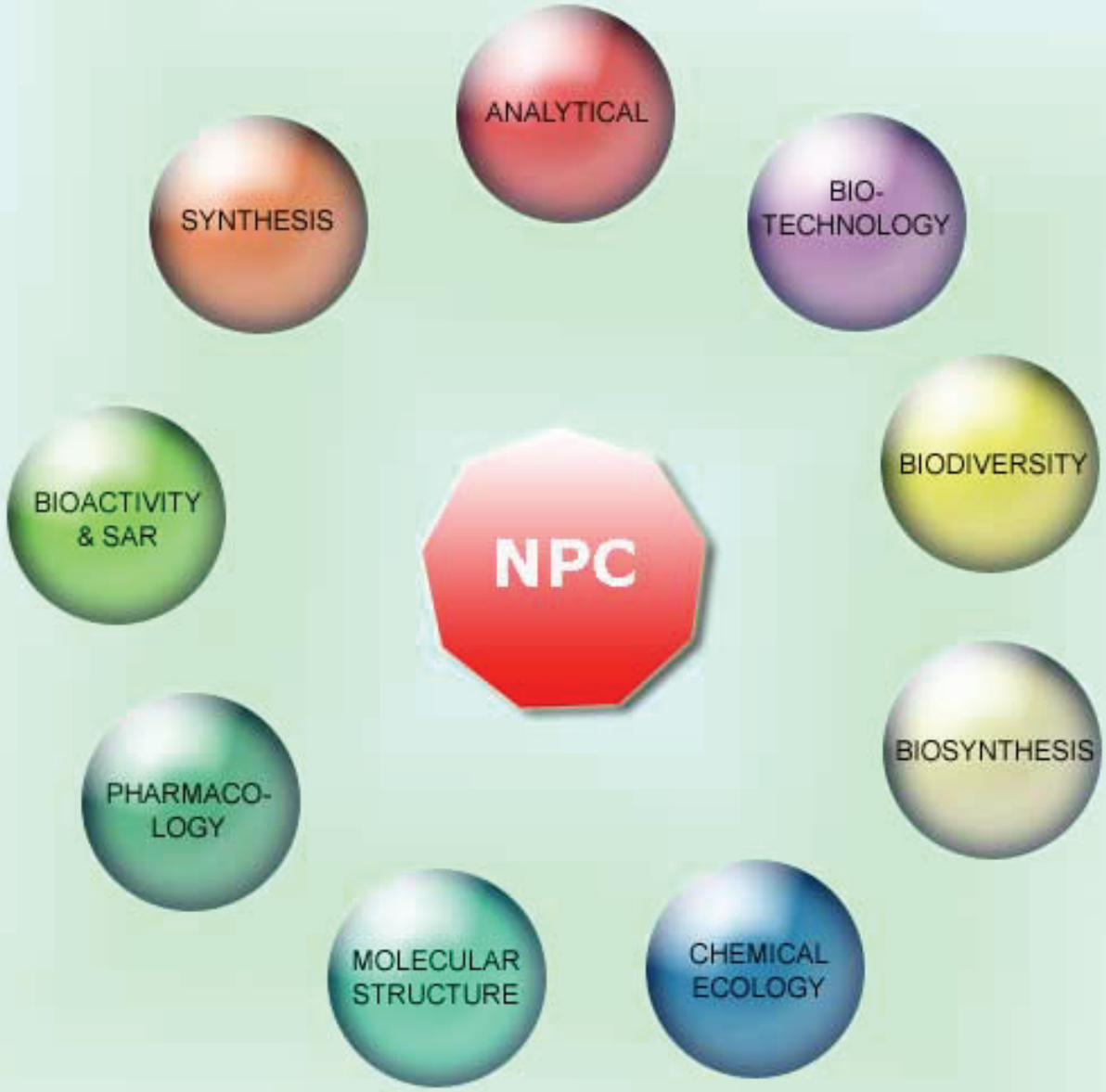

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Resveratrol Oligosaccharide Induces mRNA Expression for *SIRT*

Hiroki Hamada[a,*], Kei Shimoda[b], Yasukazu Saitoh[c], Shouta Doi[a], Yuya Fujitaka[a], Tsubasa Ono[a], Hatsuyuki Hamada[d] and Minami Araki[a]

[a]*Department of Life Science*, *Faculty of Science*, *Okayama University of Science*, *1-1 Ridai-cho*, *Kita-ku*, *Okayama 700-0005*, *Japan*

[b]*Department of Biomedical chemistry*, *Faculty of Medicine*, *Oita University*, *1-1 Hasama-machi*, *Oita 879-5593*, *Japan*

[c]*Faculty of Life and Environmental Sciences*, *Prefectural University of Hiroshima*, *562 Nanatsuka*, *Shobara*, *Hiroshima 727-0023*, *Japan*

[d]*National Institute of Fitness and Sports in Kanoya*, *1 Shiromizu-cho*, *Kagoshima 891-2390*, *Japan*

hamada@dls.ous.ac.jp (Hiroki Hamada)

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Piceid (resveratrol 3-*O*-β-D-glucoside) was glycosylated by cyclodextrin glucanotransferase to give oligosaccharides of resveratrol. Resveratrol oligosaccharides induced mRNA expression of *SIRT1-6*.

Keywords: Piceid, Cyclodextrin glucanotransferase, Oligosccharide, Expression of mRNA, Sirtuin.

Resveratrol (*trans*-3,4′,5-trihyrdoxystilbene), which has been isolated from fruits such as grapes and berries, and several medicinal plants, is one of the most important stilbene compounds [1]. It shows anti-oxidant, anti-allergic, and anti-aging (sirtuin activating) activity [2-6]. On the other hand, the glycosylation reaction enhances the solubility of the substrates, and increases the activity of biosynthetic intermediates in plant cells [7]. Recently, glycosylation of organic compounds by biocatalysts, such as UDP-glycosyltransferases, has attracted synthetic attentions, because of its simple reaction procedure compared with chemical glycosylation, which requires several procedures for protection-deprotection of sugar moiety. Furthermore, the biocatalytic glycosylation of organic compounds can improve their water-solubility, stability, and absorption after oral administration, and increase their biological activities. It is well known that glycosylation provides advantageous changes in pharmaceutical activity compared with the aglycone molecule. Herein, we report the preparation of oligosaccharides of resveratrol by glycosylation of piceid with glucosylransferase. Also, the effects of resveratrol oligosaccharides on expression of *SIRT1-7* are reported for the first time.

Piceid (resveratrol 3-*O*-β-D-glucoside, **1**) was glycosylated by cyclodextrin glucanotransferase according to previously reported procedures [8]. HPLC analysis showed that products consisted over five glycosylated compounds (Figure 1). ESI$^-$/mass analyses of products **2**-**5** indicated these compounds had 2-5 hexoses in the corresponding molecules ([M−H]$^-$ *m/z*: 551 (**2**), 713 (**3**), 875 (**4**), 1037 (**5**)), showing products are mixture of saccharides of resveratrol. The chemical structure of sugar moiety of compound **3** was determined to be β-maltotrioside [α-(1→4)-glucooligo-saccharide] by NMR spectroscopic method [8].

The cytotoxicity of resveratrol oligosaccharides was examined using normal human fetal lung fibroblast TIG-1 cells. The cells were treated with test sample at variable concentrations for 24 h. The resveratrol oligosaccharides showed low cytotoxicity (Figure 2).

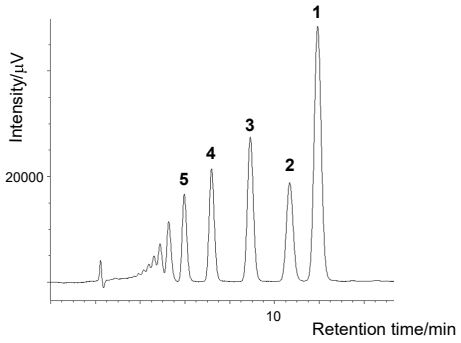

Figure 1: HPLC chromatogram of oligosaccharide products **2**-**5** from piceid (**1**).

Next, the effects of resveratrol oligosaccharides on mRNA expression for *SIRT1-7* in TIG-1 cells were examined. The TIG-1 cells were treated with 100 mg/L of resveratrol oligosaccharides for 24 h. Relative mRNA expression levels of *SIRT1-7* gene were analyzed by quantitative real-time PCR. The enhancement of mRNA expression levels for *SIRT1-6* was found in the cells treated with resveratrol oligosaccharides (Figure 3).

Thus, it was demonstrated that resveratrol oligosaccharides have positive potential for expression of *SIRT* gene in TIG-1 cells. The results obtained here clearly showed that resveratrol oligosaccharides significantly enhanced *SIRT1-6* expression in the cells. Recently, it has been reported that resveratrol is a potent activator of SIRT1 [9]. This is the first report of the positive effects of resveratrol glycosides on *SIRT* gene. Resveratrol oligosaccharides shows not only quite better water-solubility, but also quite lower cytotoxicity than resveratrol (data not shown). Therefore, cells are able to be exposed to higher concentrations of resveratrol oligosaccharides. It was suggested that higher concentration of resveratrol oligosaccharides might increase the accessibility to cells attributed to its high water-solubility, and potentiate the various effects of resveratrol due to its low cytotoxicity.

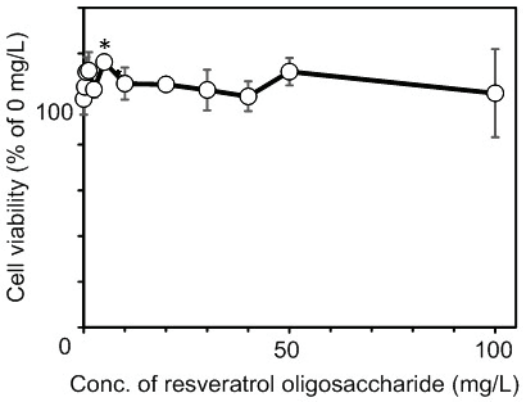

Figure 2: Cytotoxicity of resveratrol oligosaccharides in TIG-1 cells.
Results are expressed as mean ± SD (n = 3-10). Significantly different from control: *P < 0.05.

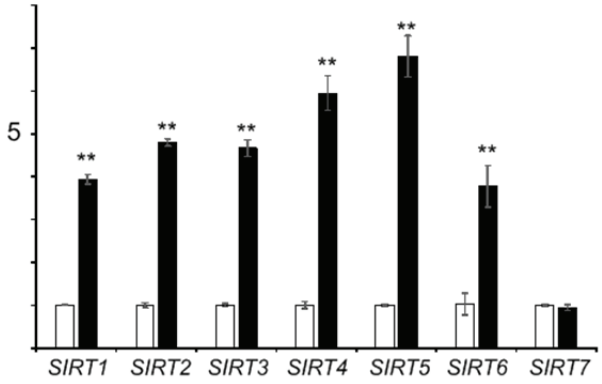

Figure 3: Differences in mRNA expression for *SIRT1-7* in TIG-1 cells.
Values are expressed as mean ± SD (n = 3). **Significantly different from control: P < 0.01. White and black boxes shows controls and results in the presence of resveratrol oligosaccharides (100 mg/L), respectively.

Experimental

Cell condition: Normal human fetal lung fibroblast TIG-1 cells were obtained from the Japanese Collection of Research Bioresources (Osaka, Japan. The cells were cultured in Eagle's minimum essential medium (MEM, Nissui Pharmaceutical Co. Ltd., Tokyo) supplemented with 10% heat-inactivated fetal bovine serum (Biological Industries, Ltd., Kibbutz Beit-Haemek, Israel) and 2 mM L-glutamine (Wako Pure Chemical Industries Ltd., Osaka, Japan) at 37°C in an atmosphere of 95% humidified air and 5% CO_2. The cells were continuously cultivated, and cellular replicative senescence was induced.

Evaluation of cell viability: Cell number was assessed based on mitochondrial enzymatic conversion of WST-1 [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2.4-disulfophenyl)-2H-tetrazolium, sodium salt] (Dojindo Laboratories, Kumamoto, Japan) to yellowish formazan, indicative of viable cells. Following AsA treatment and subsequent 23 h incubation, cells were rinsed with phenol red-free DMEM and incubated for 3 h in phenol red-free DMEM containing 5 mM WST-1 and 0.2 mM 1-methoxy-5-methylphenazinium methylsulfate at 37°C. Absorbance at 450 nm was measured with a microplate reader (FLUOstar OPTIMA; BMG Labtech, Offenburg, Germany). Cell viability was expressed as the percentage of absorbance relative to that of the non-treatment control cells.

Real-time quantitative polymerase chain reaction: Real-time, reverse transcription (RT), quantitative polymerase chain reaction (qPCR) was performed according to the manufacturer's protocols at each stage. Total RNA was extracted from cells using the NucleoSpin RNA II kit (Takara Bio Inc., Shiga, Japan). RT reactions were then performed using a PrimeScript® RT Master Mix (Perfect Real Time) (Takara Bio). Quantitative real-time RT-PCR reactions were carried out using 2 μL (50 ng) cDNA as template; 0.8 μL of the forward and reverse primers appropriate for each target gene, and SYBR® Premix Ex Taq ™ II reagent with SYBR Green (Takara Bio). Reactions were conducted using the ECO Real-Time PCR system (Illimina Inc., CA, USA) with the following reaction profile: pre-denaturation for 30 s at 95°C, followed by 40 cycles of PCR amplification at 95°C for 5 s and 60°C for 30 s. We then performed a melt curve analysis to determine the reaction specificity. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method after normalization by reference to glyceraldehyde 3-phosphate expression.

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