Solubilities of Soybean Saponins and Their Solubilization with a Bisdesmoside Saponin

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The solubilities of Bb (soyasaponin I), one of the major monodesmoside saponins in soybean seeds, were measured in aqueous ethanol and buffer. Bb showed the highest solubility in 60% ethanol, and was more soluble in alkaline region in buffers prepared at various pHs. On the other hand, Bb' (soyasaponin III) and B-G (3-O- β -D-glucuronopyranosyl soyaapogenol B) were almost insoluble in buffer. Solubilities of Bb, Bb' and B-G in buffer were increased by the addition of Ab (acetyl-soyasaponin A₁), which is one of the major bisdesmoside saponins in soybean seed hypocotyl. The solubilizing effects of bisdesmoside saponins on monodesmosides, which are responsible for some physiological effects in soybean, may be important for processing beneficial foods using saponins for human consumption.

Many kinds of saponins have been isolated and characterized from soybean seeds¹⁾⁻⁵⁾. Soybean saponins are mainly divided into two groups: monodesmoside saponins, e.g., Ba (soyasaponin V), Bb (soyasaponin I) and Bc (soyasaponin II) (group B)⁴⁾; and acetylated bisdesmoside saponins, e.g., Aa (acetyl-soyasaponin A₄), Ab and Af (acetyl-soyasaponin A₂) (group A)⁵⁾. Recently, some physiological

effects, (anti-viral⁶⁾, hypolipidemic⁷⁾ and anti-thrombic effects⁸⁾) and an undesirable taste⁹⁾ were reported. Thus, saponins have attracted much attention not only in the medicinal area but also in food processing in terms of more beneficial uses. Although saponins may exhibit these physiological effects in aqueous solution, monodesmoside saponins are generally considered to be sparingly soluble in water¹⁰⁾. Therefore, it is important to investigate the solubilities of saponins in water.

It should be mentioned that certain bisdesmoside saponins which occur in some medicinal plants such as *Hemsleya chinensis*, *Sapindus mukurossi* and *Panax japonicus*, showed solubilizing effects on monodesmoside saponins in water¹⁰⁾⁻¹³⁾. Knowledge of the effects of bisdesmosides on the solubilities of monodesmosides in soybean saponin is important in considering the utilization of biologically active saponins.

In this paper, the solubilities of monodesmoside saponins in soybean seeds are considered, especially Bb, Bb' and B-G (Fig. 1), along with the effects of one of the bisdesmoside saponins (Ab) (Fig. 1) on the solubilities of monodesmoside saponins.

Materials and Methods

Preparation of soybean saponins

Soybean saponins Bb and Ab were isolated from soybean hypocotyls according to a previous paper¹⁴⁾. Bb' and B-G were prepared in the following manner as; group B fraction extracted from soybean seed hypocotyl was partially hydrolyzed with 0.1 N HCl at 80°C for 48 h. The hydrolyzate was neutralized with NaOH and high performance liquid chromatography (HPLC) was used to isolate each constituent.

Determination of solubilities of saponins

Each exactly measured saponin (2-4 mg) was put in an Ependorf tube (1.5 ml) or a glass tube (2 ml), and then the solvent (200 μ l) was added. The mixture was vigorously shaken, and then centrifuged for 10 min at 10 000 rpm. The supernatant was injected into the chromatograph and the saponins were quantified.

Fig. 1 Structures of soybean saponins

High Performance Liquid Chromatography (HPLC)

The instruments used for saponin analyses were a Waters Model 510 pump, a Model 484 variable wavelength UV detector (205 nm) and a Waters Model 741 data module. The column used was a YMC-GEL ODS AM-303 (5 μ m, 250 $\times$ 4.6 mm i.d., Yamamura Chemical Laboratories Ltd.). The mobile phase was methanol-2-propanol-water-acetic acid = 70 : 6 : 23.9 : 0.1 (v/v/v/v), and the flow rate was 0.5 ml/min. The column used to separate Bb' and B-G preparation was a YMC-GEL ODS AM-323 (7 μ m, 250 $\times$ 10 mm i.d., Yamamura Chemical Laboratories Ltd.) and a mobile phase used was methanol-acetonitrile-1-propanol-water-acetic acid = 38 : 20 : 6 : 36 : 0.05 (v/v/v/v/v), and the flow rate was 3 ml/min.

Results and discussion

Solubility of Soybean Saponin Bb in Aqueous Ethanol

Solubilities of Bb in ethanol, which contained various proportion of water, are shown in Fig. 2. The solubility of Bb was the highest (13 mg/ml) in 60% ethanol (v/v). These data

Fig. 2 Effect of concentration of ethanol on the solubility of soybean saponin Bb

coincided with the report that saponins are extracted most effectively with 60 to 70% ethanol from soybean seeds¹⁵⁾ (extraction rate : 97% (unpublished data)). Further, in ethanol containing less than 30% water, the Bb solution showed the great turbidity, so saponins are suggested to form insoluble aggregates in water.

Effect of pH on solubility of Bb

In order to remove the turbidity of the

saponin solution, Bb was dispersed in 0.067 M phosphate buffer containing 2% NaCl. The effect of pH on Bb solubility in such a buffer is shown in Fig. 3. The solubility of Bb was very low in the acidic region, but greatly increased in the 6.5 to 7.3 pH region. The inflection point of this curve was about 7.1. In the alkaline region, solubility of Bb was relatively high.

On the other hand, the solubilities of Bb' and B-G were very low and could not be determined using the HPLC technique as with Bb (data not shown). Removal of only one sugar residue greatly decreased the solubilities of the saponins.

From these data, in the physiological pH condition, Bb in soybean saponins appears to be relatively soluble in buffer, whereas Bb' and B-G were insoluble. These solubilities, or the absence thereof, may be important for their physiological effects on the human body.

Effect of addition of soybean group A saponin on solubilities of Bb, Bb' and B-G

As described above, solubilities of soybean saponins Bb, Bb' and B-G were very low in water, and Bb' and B-G in particular were sparingly soluble in buffer containing NaCl.

Saikosaponin a is also almost insoluble in water (0.14 mg/ml), but its great solubilization with a certain bisdesmoside saponin (e.g.,

hemslosides) was reported (8.7 mg/ml at 1.0 mg/ml of hemsloside Ma2)¹⁰⁾. In soybean, bisdesmoside saponins such as Aa and Ab occur, so the effects of Ab on the solubilities of Bb, Bb' and B-G in buffer were examined. The solubility of Bb increased by about 1.5 times with Ab addition. Bb' and B-G, which were sparingly soluble in the buffer, also showed increased solubility with Ab addition, and reached 0.25 and 0.3 mg/ml, respectively.

In the case of soybean saponins, the solubilizing effect of Ab on monodesmosides was shown though the effect was weak. In intact systems, the coexistence of bisdesmoside and monodesmoside saponins in plants may be important to bring about saponin bioactivities in the human body.

The solubilizing effect of Ab was relatively low, but Ab is the saponin which occurs in food ingredients, soybean seed, and has low hemolytic¹⁶⁾ and low antimicrobia activities¹⁷⁾. Therefore, it may be preferable to other saponins from medicinal plants (e.g., *Hemsleya chinensis*, *Sapindus mukurossi* and *Panax japonicus*) for human application. It is, therefore, important to study the solubilizing effect of bisdesmosides in soybean on water-insoluble compounds which have important biological activities.

Fig. 3 Effect of pH on the solubility of soybean saponin Bb

Fig. 4 Solubilizing effect of soybean saponin Ab on monodesmoside saponins
●, Bb; ○, Bb'; ▲, B-G

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大豆サポニンの溶解度およびビスデスモシドサポニンによる可溶化

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大豆種子に含まれるサポニンのうち、モノデスモシドサポニン (Bb, Bb', B-G) の含水エタノール、緩衝液中での溶解度を測定した。含水エタノール中での Bb 溶解度は 60% エタノール (v/v) の時に最も高かった。Bb 溶解度に対する pH の影響について調べた結果、酸性側では低く、アルカリ側で比較的高く、pH 7.1 付近に変曲点をもった溶解度曲線を示した。一方、Bb', B-G は検出限界以下で、緩衝液に対する溶解度は極めて低いことがわかった。この緩衝液にビスデスモシドサポニンである Ab を添加したところ、Bb のみならず、Bb', B-G においても溶解度の上昇が見られた。