Simple and Rapid Separation of Soyasaponin Bb from a Soy Extract

Hajime Katano,*† Nobuhiro Okamoto,* Masahiro Takakuwa,* Shu Taira,* Taiho Kambe,** and Masakazu Takahashi*

*Department of Bioscience, Fukui Prefectural University, Eiheiji, Fukui 910-1195, Japan
**Division of Integrated Life Science, Graduate School of Biostudies, Kyoto University, Kyoto 606-8502, Japan

A simple method to separate soyasaponin Bb from a soy extract is presented. This method is based on the difference in the solubility of soyasaponin Bb and Ba and other components into 3:7 and 1:1 (v/v) acetone-water mixed solvents. The crude soyasaponin consisting of soyasaponins Aa, Ab, Ba, and Bb at the 10 wt% level and other components was examined as the soy extract. A 10 mg quantity of the crude soyasaponin was mixed with 1 mL of the 3:7 acetone-water containing 0.1 mol/L HCl, and the supernatant was removed to obtain a precipitate, which was found to contain mainly soyasaponins Bb and Ba. The precipitate was mixed with 0.4 mL of the 1:1 acetone-water containing 0.1 mol/L HCl; the supernatant was transferred, and was mixed with 0.6 mL of water to obtain a precipitate, which was found to contain mainly soyasaponin Bb. The yield was ca. 30%, which may be much higher than that by the conventional preparative chromatographic approach. The separation method is rapid and easy to carry out, and is useful for the preparation of a soyasaponin Bb sample.

Keywords Separation, purification, soyasaponin Bb, soy extract, organic-water mixed solvent

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Introduction

In previous papers,¹² a method for the separation of basic oligo- and polypeptides was presented. The method is based on the precipitation of the polycationic species with an analytical reagent from water and the re-precipitation from an organic–water mixed solvent. Using this method, a specific ε-poly-L-lysine has been separated and purified from a culture broth.⁵ Also, oligochitosan can be separated and purified from the crude product by precipitation from water and re-precipitation from a mixed solvent.⁴ These separation methods using organic-water mixed solvent are rapid and easy to carry out, and can be applied to the purification of a natural product more advantageously than the conventional preparative liquid-chromatographic approach.

Soyasaponins have been the subject of research interest in its biological activities and potential health-beneficial effects, including anti-inflammatory, anti-carcinogenic, and tissue protective activities.⁵⁴ Although many types of soyasaponins including group A and B molecules are found in soybeans, soyasaponin Bb (Fig. 1) is the most intensively studied type because of its abundance in soybeans and its significant biological activities.⁵⁶ Moreover, bioavailability of group B soyasaponins is better than that of group A soyasaponins.¹⁰

The experimental sample of soyasaponin Bb has been separated and purified from soy products by extraction and preparative chromatographic approaches.⁰¹¹³ A commercially available crude soyasaponin was found to contain 4 major types of soyasaponins, i.e. soyasaponins Aa, Ab, Ba, and Bb, together with some impurities. Even if the crude soyasaponin was used as the starting material in the conventional separation methods, the yield of soyasaponin Bb was very poor.

In this study, we developed a simple and rapid method for the separation of soyasaponin Bb from the crude soyasaponin. Our separation method is based on the difference in the solubility of soyasaponins Bb and Ba and other components into acetone-water mixed solvent. When the crude product was mixed with the 3:7 (v/v) acetone-water, soyasaponins Bb and Ba were not dissolved in the solvent. On the other hand, almost all other components were soluble into the mixed solvent. Thus, soyasaponin Bb and Ba have been easily separated from the starting material. Soyasaponin Ba is a soyasaponin Bb analogue, of which the L-rhamnose moiety is exchanged by glucose. Thus, their chemical properties would be very similar to each other. Also, their solubility becomes complex when both exist. However, it was found that soyasaponin Bb is dissolved in the 1:1 (v/v) acetone-water, soyasaponins Bb and Ba were not dissolved in the solvent. On the other hand, almost all other components were soluble into the mixed solvent. Thus, soyasaponin Bb and Ba have been easily separated from the starting material.

Experimental

Soyasaponins Ba and Bb were obtained from Funakoshi Co., Ltd., and were used as standard materials. A crude soyasaponin was purchased from Fuji Oil Co., Ltd., and used as the starting material. Other chemicals were of reagent grade, and were used...
The treated samples were analyzed by a capillary electrophoresis (CE) technique. A CE system (Otsuka Electronics, CAPI-3300) was used in the analysis. The 0.02 mol/L Na₂B₄O₇ aqueous solution (pH 9.2) was used for a running buffer solution. The applied voltage was held at 30 kV. The column was a fused-silica capillary with a total length of 75 cm and an effective length of 52 cm (75 μm i.d.). The temperature of the capillary cartridge during electrophoresis was maintained at 25°C. Sample solutions were loaded by hydrostatic injection (25 mm, 30 s). Detection was achieved with UV absorbance at 210 nm (A₂10).

The samples were also analyzed by an HPLC technique. An HPLC system (Shimadzu, Prominence) was used in the analysis. A Cosmosil 5C₁₈-AR-II column (4.6 mm i.d. × 150 mm) was used for separation at 30°C. An aqueous solution and an acetonitrile solution were used for mobile phases A and B, respectively. Both phases contained 0.05% (v/v) trifluoroacetic acid. The A/B mixing ratio was at 95:5 for 5 min, shifted to 70:30 at 5 min, and was varied linearly to 45:55 at 30 min, consecutively to 0:100 for column washing. The flow rate was kept constant at 1 mL/min throughout the experiment. Detection was also achieved with UV absorbance at 210 nm, A₂10.

Results and Discussion

CE of standard material of soyasaponins Bb and Ba
A 1 mg quantity of the standard material of soyasaponin Bb was dissolved completely in 1 mL of the 0.02 mol/L Na₂B₄O₇ aqueous solution (pH 9.2), although soyasaponin Bb is hardly soluble in water. As shown in Fig. 1, soyasaponin Bb has one carboxyl group and two cis-diol groups, which react with borate anion to form a negatively charged ester.¹⁴⁻¹⁶ In the borax solution, the borate anion would be formed according to the following reaction: B₄O₇⁻ + 7H₂O = 2B(OH)₄⁻ + 2B(OH)₃.¹⁷ Therefore, soyasaponin Bb would exist as a trivalent anionic species in the borax solution. Figure 2 shows an electropherogram of the 1 g/L soyasaponin Bb in the 0.02 mol/L Na₂B₄O₇ aqueous solution. A well-developed peak was observed at around 5.4 min.

When 1 mg of the standard material of soyasaponin Ba was mixed with 1 mL of the 0.02 mol/L Na₂B₄O₇ aqueous solution, a precipitate was observed remarkably in the mixture. No elution peak was found in the electropherogram of the supernatant, indicating very low solubility of soyasaponin Ba in the borax solution. This may be due to that soyasaponin Ba has only one cis-diol group. However, the elution peak for soyasaponin Ba was observed in the electropherogram given by

Fig. 1  Chemical structure of soyasaponins Aa, Ab, Ba, and Bb.
a mixed precipitate of soyasaponins Bb and Ba (Fig. 3B below). Because of their surface active properties,13,18–22 soyasaponin Ba would be dissolved or dispersed into the borax solution in the presence of soyasaponin Bb.

**CE and HPLC of starting material**

A 10 mg quantity of the crude soyasaponin was mixed with 1 mL of the 0.02 mol/L Na₂B₄O₇ aqueous solution. After centrifugation, a precipitate was found significantly in the mixture, suggesting that some components cannot be dissolved in the borax solution. However, as shown by Fig. 3A, the supernatant gave many well-developed impurity peaks, and soyasaponin Bb could not be detected clearly in the electropherogram. Although the experimental condition was modified, the elution peaks were not separated successfully. However, because the elution peak for soyasaponin Bb is observed within several minutes, the CE analysis was useful for screening the optimum separation condition, as can be seen in Fig. 6 below.

A 10 mg quantity of the crude soyasaponin was mixed with 1 mL of MeOH, and the mixture was centrifuged. After centrifugation, a very small amount of precipitate was found in the mixture, suggesting that many components would be dissolved into MeOH. As shown by Fig. 4A, many elution peaks are observed in the chromatogram of the MeOH solution.

Using standard materials, the elution peaks for soyasaponins Aa, Ab, Ba and Bb were assigned as indicated in the figure, and their concentrations in the MeOH solution were determined to be 1.5 ± 0.3, 2.0 ± 0.3, 0.8 ± 0.1, and 1.9 ± 0.2 g/L, respectively. Thus, it was estimated that the crude soyasaponin contained 19 wt% soyasaponin Bb, 8 wt% soyasaponins Ba, and same concentration level soyasaponins Aa and Ab.

**Separation of soyasaponins Bb and Ba from the starting material**

The procedure of the present separation method is illustrated in Fig. 5. In the 1st operation, 10 mg of the starting material was mixed with 0.3 mL acetone, 0.6 mL of water, and 0.1 mL of the 1 mol/L HCl aqueous solution, that is, 1 mL of the 3:7 (v/v) acetone-water mixed solvent containing 0.1 mol/L HCl; the mixture was centrifuged, and the supernatant was removed to obtain a precipitate (ppt-1).

The ppt-1 precipitate was dissolved into 1 mL of the 0.02 mol/L Na₂B₄O₇ aqueous solution. Figure 3B shows an electropherogram of the aqueous solution. A well-developed peak was observed at around 5.4 min, indicating that ppt-1
contained soyasaponin Bb. However, a shoulder peak was also observed at around this time. A nano LC-MS analysis of ppt-1 showed well-developed signals at m/z 943 and 959, which correspond to soyasaponins Bb and Ba, respectively. Thus, the shoulder peak can be assigned to be soyasaponin Ba. However, surprisingly, other impurity peaks were decreased remarkably, indicating that other components were successfully removed.

Figure 4B shows a chromatogram of the MeOH solution, of which ppt-1 was dissolved into 1 mL of the solvent. Well-developed elution peaks for soyasaponins Bb and Ba were observed, and other peaks were decreased remarkably. The results indicate again that soyasaponins Bb and Ba were successfully separated from the starting material. This can be due to the lower solubility of soyasaponins Bb and Ba and the high solubility of other components into the 3:7 acetone-water mixed solvent. It is noted that soyasaponins Bb and Ba would exist as free acid forms because of the presence of HCl. When the 3:7 acetone-water solution was non- or alkaline buffered, larger amounts of impurities were detected from ppt-1.

When the 4:6 acetone-water was used instead of the 3:7 acetone-water, larger amounts of impurities were found remarkably from ppt-1. When the 2:8 acetone-water was used, a smaller amount of soyasaponin Bb was detected. Also, the 1st operation was performed using MeOH, EtOH, 2-propanol, ethylene glycol, acetonitrile, and dimethyl sulfoxide, instead of acetone. Figure 6 shows electropherograms given by the ppt-1 precipitates. Larger amounts of impurities were found from ppt-1, indicating that the solubilities of the impurity components in the mixed solvents are lower than those in the acetone-water. Thus, although the peak area for soyasaponin Bb was decreased to 70% of that for the starting material, the use of 3:7 acetone-water is the most preferable.

At present, the difference in the solubility of soyasaponins Bb and Ba and other components into these organic-water mixed solvents cannot be explained by using the physicochemical properties, such as the dielectric constant and the donor-acceptor numbers, of the organic solvents. A solution chemical study of soyasaponin Bb should be needed in the future.

Separation of soyasaponin Bb from the mixed precipitate with soyasaponin Ba

To obtain further purified soyasaponin Bb, the 2nd operation was performed as described in Fig. 5. The ppt-1 precipitate was mixed with 0.2 mL of acetone and 0.2 mL of the 0.2 mol/L HCl aqueous solution, that is, 0.4 mL of the 1:1 (v/v) acetone-water containing 0.1 mol/L HCl; the mixture was centrifuged; the supernatant was transferred, and was mixed with 0.4 mL of water; the mixture was centrifuged, and the supernatant was removed to obtain a precipitate (ppt-2).

The ppt-2 precipitate was dissolved into 1 mL of the 0.02 mol/L Na2B4O7 aqueous solution. Figure 3C shows an electropherogram of the borax solution. The shoulder peak and impurity peaks are no longer observed significantly, indicating that soyasaponin Ba and other impurities were further removed. Figure 4C shows a chromatogram given by ppt-2. The peak area of soyasaponin Bb was decreased to ca. 40% of that for ppt-1, indicating that soyasaponin Bb was not completely dissolved into the 1:1 acetone-water. However, the ratio of the peak area of soyasaponin Ba to soyasaponin Bb decreased to ~0.1, indicating a lower solubility of soyasaponin Ba in the mixed solvent, even in the presence of soyasaponin Bb.

When 0.4 mL of the 2:1 acetone-water and 0.8 mL of the 1:1 acetone-water was used instead of 0.4 mL of the 1:1 acetone-water, soyasaponin Ba was found remarkably from the resulting ppt-2. Also, the 2nd operation was performed using 0.2 mL of the 1:1 acetone-water. Soyasaponin Ba was also removed to some extent, but the yield of soyasaponin Bb became lower than that when 0.4 mL of the 1:1 acetone-water was used. Thus, the use of 0.4 mL of 1:1 acetone-water is the most preferable at present.

By comparing the peak area of soyasaponin Bb in Fig. 4C with that in Fig. 4A, the yield of soyasaponin Bb from the starting material was calculated to be ca. 30%. By comparing the peak area in Fig. 4C, it can be considered that ppt-2 contained soyasaponin Bb at ca. 90 wt%. Thus, ppt-2 can be used as a soyasaponin Bb sample in some experiments. Also, a more high-purity soyasaponin Bb sample could be prepared by the column separation of ppt-2.

The present method is simple, rapid, and easy to carry out. In conclusion, the present separation method is useful for the separation and purification of soyasaponin Bb from crude soyasaponin. The study is being extended to separation from other natural resources.

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