Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 63 (2003) 817–823
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Size exclusion chromatographic analysis of polyphenol–serum albumin complexes

Tsutomu Hatano[a,*], Mami Hori[a], Richard W. Hemingway[b], Takashi Yoshida[a,*]

[a]*Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700-8530, Japan*
[b]*Plant Polyphenols LLC, PO Box 12522, Alexandria, LA 71315, USA*

Received 13 February 2003; received in revised form 9 April 2003

Abstract

Formation of water-soluble polyphenol–protein complexes was investigated by size-exclusion chromatography (SEC). The combination of (−)-epigallocatechin gallate (EGCG) and bovine serum albumin (BSA), which did not form a precipitate after the solutions were mixed, showed an SEC peak due to complex formation 2–24 h after mixing. Peak size of the complex varied with time, suggesting slow change of the conformation of the protein accompanied by complexation. Formation of the complex was substantiated by ultrafiltration of the mixture; the complex did not pass through a membrane with a 100,000 nominal molecular weight limit (NMWL). The SEC profile varied with the combination of compounds. The peaks due to the complexes showed that the apparent value of the number average molecular weight (M_n) of the EGCG–BSA complex was 2.8×10^5, while that of a pentagalloylglucose (PGG)–BSA complex was 9.5×10^5 under the conditions used. Dimeric hydrolyzable tannins, oenothein B and cornusiin A, also caused changes in the SEC profile of BSA, although the combinations did not show peaks attributable to formation of such large complexes observed for EGCG and PGG. Procyanidin B3 and (+)-catechin did not cause changes in the SEC profile of BSA. With cytochrome *c*, EGCG did not show any chromatographic changes.

Keywords: Polyphenol; Tea; *Camellia sinensis*; Theaceae; Epigallocatechin gallate; Pentagalloylglucose; Proanthocyanidin; Hydrolysable tannin; Serum albumin; Cytochrome *c*; Complexation

1. Introduction

As revealed in structural studies (Okuda et al., 1995; Yoshida et al., 2000), natural polyphenols have a wide variety of biochemical and pharmacological properties. These include host-mediated antitumor effects, antiviral effects, and the suppression of antibiotic-resistance of bacteria (Shimizu et al., 2001). These effects are, at least in part, due to interactions between polyphenols and biomolecules, mediated by hydrogen bonding, hydrophobic interactions, and covalent bonding (Haslam, 1989, 1998). Recent papers have shown the positions of interacting polyphenol and peptide molecules in solution using ^{1}H NMR spectroscopy (Baxter et al., 1997; Richard et al., 2001; Wroblewski et al., 2001). The size of polyphenol–protein complexes has also been analyzed (Charlton et al., 2002).

The polyphenol interaction, utilized for tanning in the leather industry, produces insoluble complexes, which is often used to estimate the quantities of polyphenols in plant extracts (Scalbert, 1992; Kawamoto and Nakatsubo, 1997). However, the precipitability of these complexes is dependent on the solubility of the constituent polyphenols and proteins (Takechi and Tanaka, 1987); indeed, some complexes of polyphenols and proteins are soluble.

The tea polyphenol (−)-epigallocatechin gallate **1** (EGCG) appears in blood after oral administration to mammals and humans (Unno et al., 1996; Nakagawa et al., 1997; Zhu et al., 2000), although EGCG **1** catabolism by intestinal bacterial flora prior to absorption was also reported (Kohri et al., 2001; Meng et al., 2002). Serum albumins that transport various compounds in blood are thought to form soluble complexes with polyphenols, the possible functions of which have also been studied (Riedl and Hagerman, 2001).

* Corresponding authors. Tel. +81-86-251-7937; fax: +81-86-251-7936.
E-mail addresses: hatano@pharm.okayama-u.ac.jp (T. Hatano), yoshida@pharm.okayama-u.ac.jp (T. Yoshida).

0031-9422/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0031-9422(03)00340-6

The aim of this study is to visualize the formation of water-soluble complexes by size exclusion chromatography (SEC), using EGCG **1** and bovine serum albumin (BSA), or related polyphenol–protein combinations. While SEC is widely used to estimate the molecular size of proteins, the behavior of water-soluble, protein–polyphenol complexes in SEC analyses is expected to be similar to that of the proteins. This is because the protein molecules involved are much bigger than the polyphenol molecules. Therefore, we used SEC columns, which are available for protein analysis, to analyze polyphenol–protein complexes.

2. Results and discussion

2.1. Solubility of polyphenol–protein combinations

To find water-soluble polyphenol–protein combinations adequate for the SEC analysis, precipitation tests were conducted with some polyphenols before the SEC analyses, although combinations that do not produce precipitates do not necessarily form complexes. Weakly acidic (pH 4) and neutral (pH 7) buffers were used as solvents. Alkaline conditions were not used because polyphenols are labile at alkaline pH. Three flavanoids, EGCG **1**, (+)-catechin **2** and procyanidin B3 **3**, and four hydrolyzable tannins, pentagalloylglucose **4** (PGG), corilagin **5**, oenothein B **6** and cornusiin A **7**, were used as polyphenols, and BSA and cytochrome *c* as proteins. The results were summarized in Table 1.

- Polyphenols and BSA: Of the flavanoids tested, EGCG **1** (0.125 mg/ml) gave precipitates at pH 4, but not at pH 7 even at a higher concentration (0.5 mg/ml). (+)-Catechin **2** and procyanidin B3 **3** (each at 0.5 mg/ml) did not form precipitates at either pH 4 or pH 7. Of the hydrolyzable tannins tested, PGG **4** (0.125 mg/ml) formed precipitates at pH 4 and pH 7, and corilagin **5** precipitated with the concentration of 0.125 mg/ml at pH 4 and with 0.25 mg/ml at pH 7. Oligomeric hydrolyzable tannins (oenothein B **6** and cornusiin A **7**) gave precipitates with the concentration of 0.125 mg/ml at pH 4, but not with 0.5 mg/ml at pH 7.
- Polyphenols and cytochrome *c*: EGCG **1** gave precipitates with cytochrome *c* at 0.125 mg/ml and pH 4, and at 0.25 mg/ml and pH 7. (+)-Catechin **2** did not have any effect at 0.5 mg/ml and pH 4, while it formed precipitates at 0.125 mg/ml and pH 7. Procyanidin B3 **3** (0.5 mg/ml) did not form precipitates at either pH 4 or 7. All of the hydrolyzable tannins tested (**4**–**7**) at 0.125 mg/ml formed precipitates with cytochrome *c* at pH 4 and at pH 7. These results suggest that using BSA is more adequate for the SEC analyses of soluble complexes.

Table 1
Mimimum concentrations of polyphenols required for precipitating proteins

Polyphenol	MCP_{BSA} (mg/ml)[a]		MCP_{cytc} (mg/ml)[b]	
	pH 4	pH 7	pH 4	pH 7
Flavanoids				
EGCG **1**	<0.125	>0.5	<0.125	0.25
(+)-Catechin **2**	>0.5	>0.5	>0.5	<0.125
Procyanidin B3 **3**	>0.5	>0.5	>0.5	>0.5
Hydrolyzable tannins				
PGG **4**	<0.125	<0.125	<0.125	<0.125
Corilagin **5**	<0.125	0.25	<0.125	<0.125
Oenothein B **6**	<0.125	>0.5	<0.125	<0.125
Cornusiin A **7**	<0.125	>0.5	<0.125	<0.125

[a] Minimum concentration required for precipitating BSA (0.5 mg/ml) within 2 days after adding polyphenols.

[b] Minimum concentration required for precipitating cytochrome *c* (0.5 mg/ml) within 2 days after adding polyphenols.

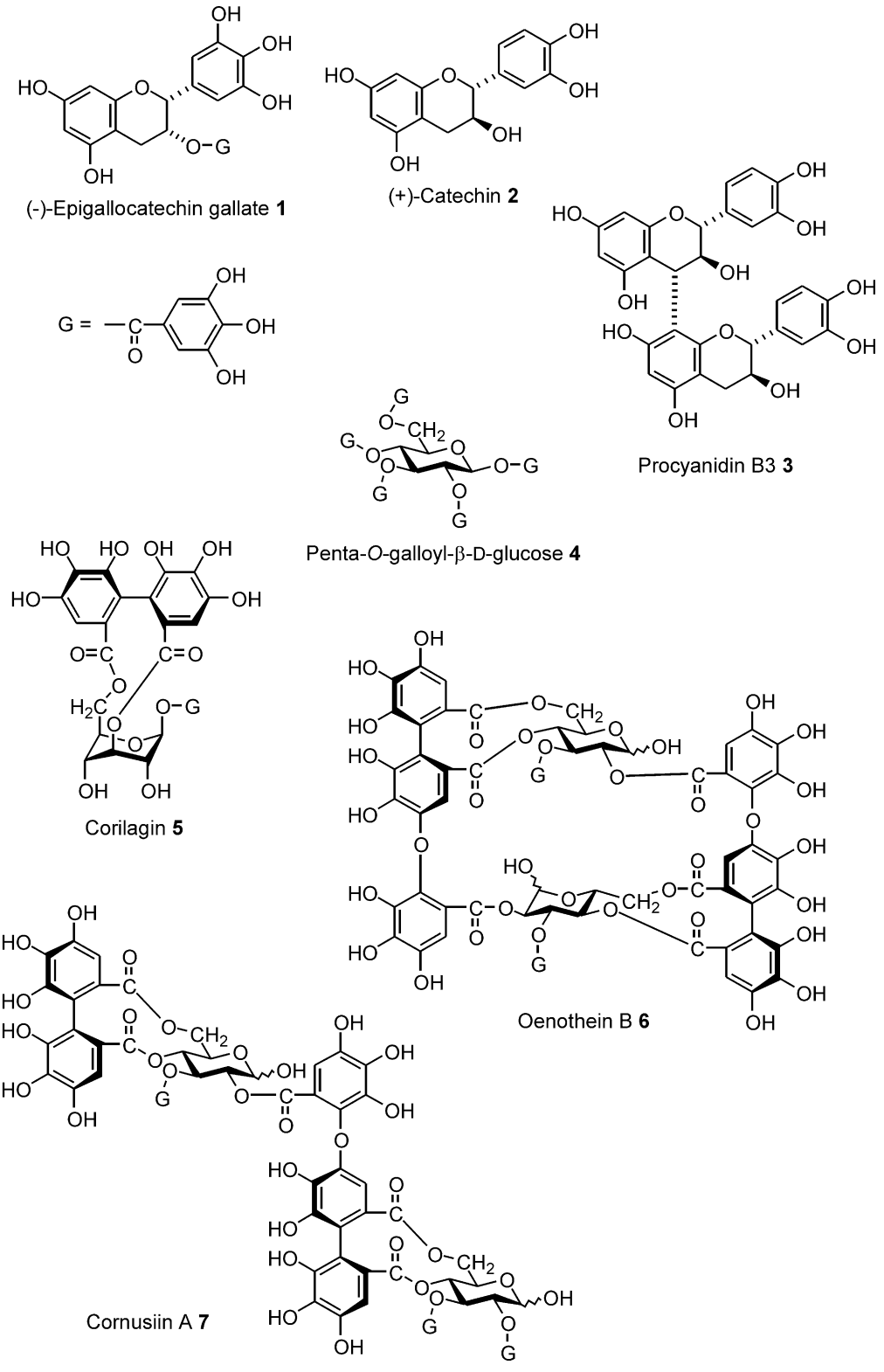
2.2. Size exclusion chromatography of polyphenol–BSA complexes

Since the SEC analyses of proteins can be conducted in solvents at around pH 7 as the mobile phase, the precipitation tests at pH 7 indicated that SEC could be used to analyze the polyphenol–BSA combinations. Although PGG **4** gave precipitates with BSA even at 0.125 mg/ml, the supernatant after the precipitation was also analyzed with SEC in order to know whether some soluble complexes are present or not. A hydrophilic silica gel column (YMC Diol-300), which is used to estimate the molecular weights of proteins up to about 1×10^6 Da, was mainly used to analyze the complexation of polyphenols with BSA.

2.2.1. Analyses of the combinations of EGCG 1 and BSA

BSA showed one major peak, along with minor peaks due to dimeric and trimeric forms [Fig. 1(a)]. By contrast, the polyphenolic EGCG **1** did not show clear peaks (data not shown), perhaps due the ionization of its phenolic hydroxyl groups at the pH of the mobile phase, which may have caused some interaction with the column packing material.

The peaks with the combination of EGCG–BSA were noticeably different from those of BSA on the SEC chromatogram. A peak [retention time (t_R) 11.7 min] attributed to the soluble complex of EGCG **1** and BSA was observed around 2 h after mixing the polyphenol–BSA solutions, and it gradually increased in size until 24 h after mixing [Fig. 1(b)–(d)]. The retention time 11.7 min of the peak on SEC at 24 h after mixing was distinctively shorter than that of BSA (t_R 13.8 min); the shorter retention time is consistent with the formation of molecule species much larger than BSA. No peak was observed around this region (t_R 11.7 min) for solutions lacking BSA, even when the analysis was conducted 24 h after the dissolution of EGCG **1** (data not shown). On the other hand, the SEC chromatogram of the EGCG–BSA



mixture showed peaks on the longer t_R region than that of BSA. The peaks on the longer t_R region were assignable to some degradation products from EGCG **1**.

The SEC peak attributable to the complex was also observed on a column of TOSOH G4000SW$_{XL}$, not only on the YMC diol column. Fig. 2 shows the chromatographic profile 24 h after mixing of EGCG **1** and BSA solutions on the TOSOH column.

In order to verify that the retention time of the observed peak attributed to the polyphenol–BSA complex (mixture of complexes) reflects the size of the complex, the mixture was analyzed by SEC after

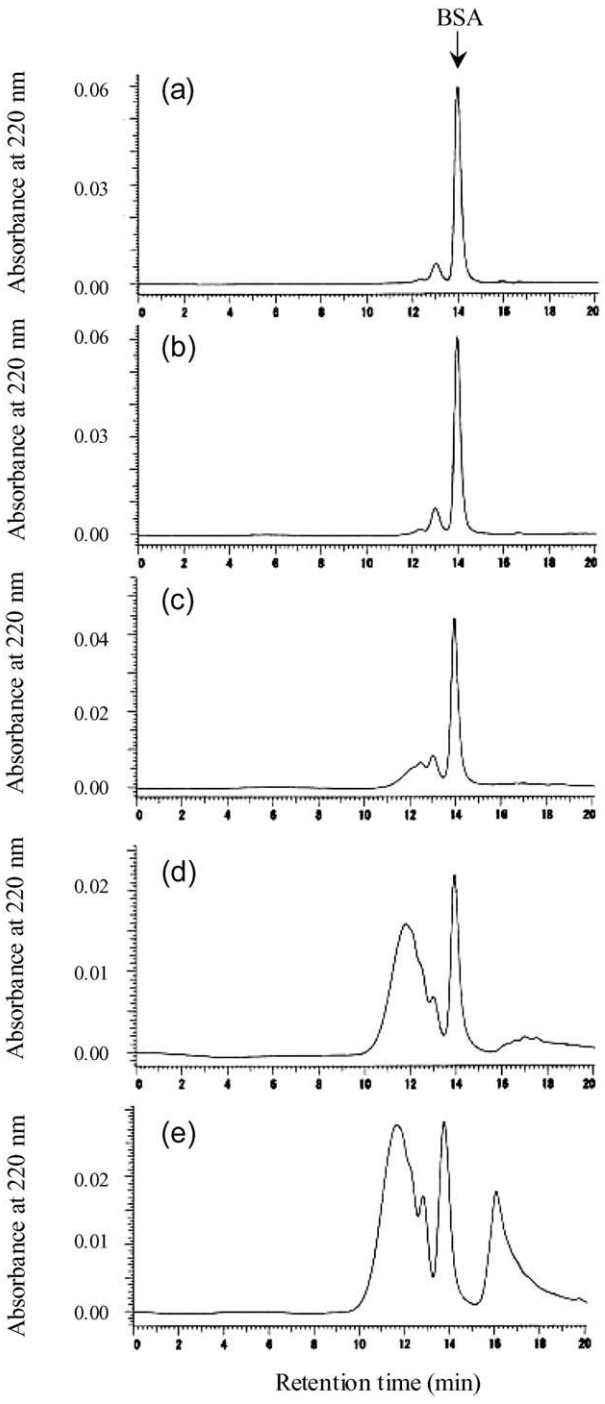


Fig. 1. Effect of the addition of EGCG **1** on the SEC profiles of BSA on a YMC Diol-300 column. The SEC profile of BSA was shown in the lane (a), and the peaks at around t_R 13 and 12.4 min were assigned to that of BSA dimer and trimer. Change in the SEC profile of the solution (b) 0, (c) 2, (d) 6, and (e) 24 h after mixing EGCG **1** and BSA were shown.

ultrafiltration. The solution containing EGCG **1** and BSA was subjected to ultrafiltration on a Biomax membrane with a 100-K nominal molecular weight limit (NMWL) (Millipore) 24 h after mixing. The amounts of complex remaining on the membrane and in the filtrate

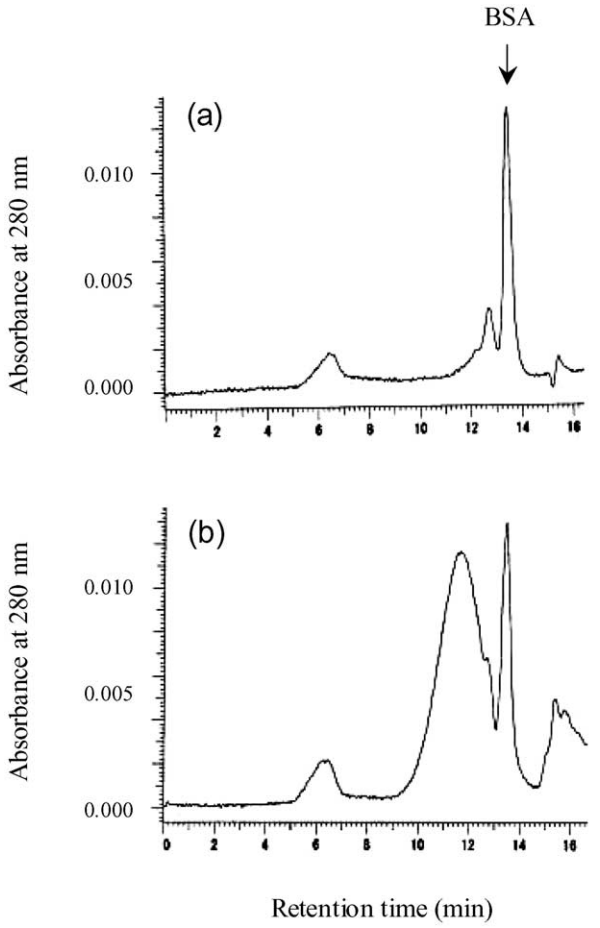


Fig. 2. SEC profile on a TOSOH G4000SW$_{XL}$ column of the solution (a) 0 h and (b) 24 h after mixing EGCG **1** and BSA. The arrow indicates the retention time of BSA.

were estimated from the peak area of the complex on the SEC chromatograms. The relative concentration of the complex in the two phases was ca. 100:1 (upper/lower phase of the membrane). This result means that almost all of the complex remained on the upper phase of the membrane, and suggested that the size of the complex exceeded 1×10^5 Da as the protein size.

The apparent molecular size based on the SEC profiles was then estimated under the assumption that this profile reflects the molecular weight: The number average molecular weight (M_n) and weight average molecular weight (M_w) estimated from the SEC peak for the EGCG–BSA complex were 2.8×10^5 and 3.5×10^5, respectively. This M_n value is corresponding to the sum of the molecular weights of four molecules of BSA.

Slow formation of the peak assigned to the complex is explainable by change in the conformation of BSA such as unfolding and folding processes, which are accompanied by complexation with EGCG **1**. On the other hand, structural change in EGCG **1** oxidation during the incubation such as dimerization to form theasinensins A and D may participate in the complexation with BSA.

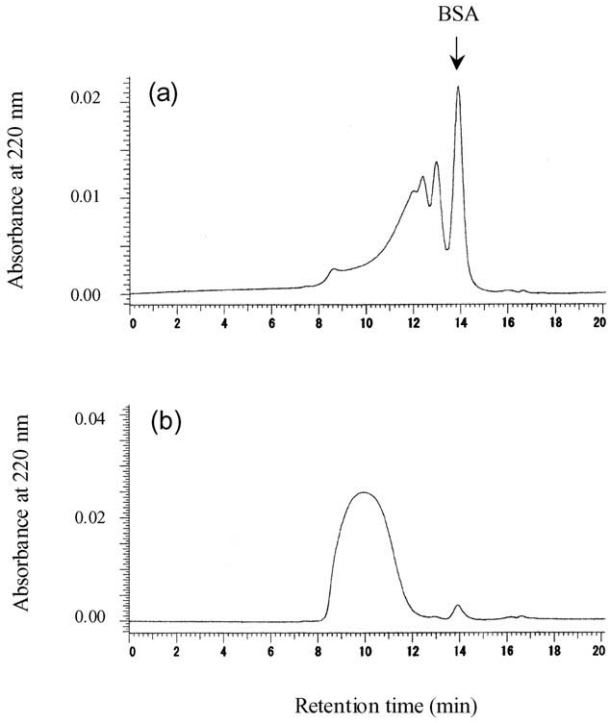


Fig. 3. SEC profiles on a YMC Diol-300 column of the solution (a) 2 and (b) 24 h after mixing PGG **4** and BSA. The arrow indicates the retention time of BSA.

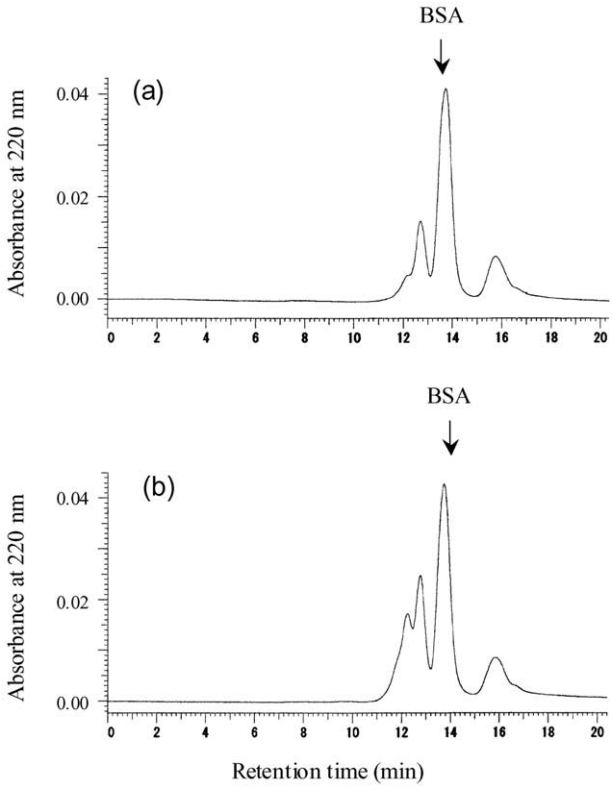


Fig. 4. SEC profiles on a YMC Diol-300 column of solutions of (a) oenothein B **6** and BSA and (b) cornusiin A **7** and BSA 24 h after mixing. The arrows indicate the retention time of BSA.

No chromatographic change attributable to complex formation was observed in combinations with cytochrome *c* (data not shown). In this case, the polyphenolic sorption may be sufficient to prevent cytochrome *c*–polyphenol complexes from eluting on this column. However, the weakness of the binding property of cytochrome *c* with polyphenols, relative to that of BSA, was suggested by the reported data on the competitive experiments for the complexation (Hagerman and Butler, 1981).

2.2.2. SEC Analyses of the combinations of other polyphenols with BSA

The combinations of catechin–BSA and procyanidin B3–BSA did not result in any changes in the BSA peak on SEC, even 24 h after mixing the polyphenol and BSA solutions. Corilagin **5**, a hydrolysable tannin, did not change the SEC profile of BSA, while PGG **4** showed a noticeable change due to complexation with BSA.

For PGG **4**, after the precipitates were removed by centrifugation, the supernatant solution was analyzed by SEC. The SEC chromatogram of the water-soluble PGG–BSA complex, which was obtained 24 h after mixing (Fig. 3), indicated that M_n and M_w of the complex (mixture) were 9.5×10^5 and 1.4×10^6, respectively. Since the SEC data were just for water-soluble parts of the complex, these data do not represent whole (soluble + insoluble) complex. However, the formation of such large complex with PGG **4** can be explained by the fact that the PGG **4** molecule has five galloyl groups, each of which can bind with a protein molecule more freely than the galloyl and hexahydroxydiphenoyl groups in a corilagin **5** molecule.

By contrast, the oligomeric hydrolyzable tannins oenothein B **6** and cornusiin A **7** did not form distinct peaks indicating formation of large complexes as observed for EGCG **1** and BSA. However, there was broadening of the BSA peak for oenothein B **6** and some increase in the peak area in the BSA–dimer or trimer region for cornusiin A **7** (Fig. 4), and it was difficult to estimate the molecular sizes of these polyphenol complexes. The spatial locations of the galloyl and other phenolic acyl groups in these polyphenol molecules are probably more restricted than those in PGG **4**.

3. Conclusions

The SEC analysis of BSA after mixing with EGCG **1** in a neutral buffer on a YMC diol-300 column showed distinctive change of the chromatographic profile, to give a new peak attributable to the polyphenol–protein complex. Peak size of the complex became larger gradually through 2–24 h after mixing, suggesting slow change of the conformation of the protein accompanied by complexation. Formation of the complex was also

supported by SEC on a TOSOH G4000SW$_{XL}$ gel column and by ultrafiltration of the mixture.

The SEC profile of the polyphenol–protein mixtures varied with the combination of compounds. The peaks due to the complexes showed that the apparent value M_n of the EGCG–BSA complex was 2.8×10^5, while that of a PGG–BSA complex was 9.5×10^5 under the conditions used. Dimeric hydrolyzable tannins, oenothein B **6** and cornusiin A **7**, also caused changes in the SEC profile of BSA, although the combinations did not show peaks attributable to formation of such large complexes observed for EGCG **1** and PGG **4**. Procyanidin B3 **3** and (+)-catechin **2** did not cause changes in the SEC profile of BSA. With cytochrome *c*, EGCG **1** did not show any chromatographic changes.

Although further confirmation of the molecular sizes assigned on the basis of the SEC profiles of the polyphenol–protein complexes by some other methods is required, these results suggested that SEC is expected to be a simple and useful method for estimating the formation of soluble polyphenol–protein complexes.

4. Experimental

4.1. Materials

BSA, cytochrome *c*, thyroglobulin, ovalbumin, myoglobin, ribonuclease A, and (+)-catechin **2** were purchased from Sigma. (−)-Epigallocatechin gallate **1** was isolated from leaves of *Camellia sinensis* (Okuda et al., 1990). PGG **4** was prepared by treating tannic acid (gallotannin mixture) in MeOH containing acetate buffer (pH 6) (Hatano et al., 1990a). Procyanidin B3 **3** was prepared from taxifolin and (+)-catechin (Hatano and Hemingway, 1997). Oenothein B **6** and cornusiin A **7** were isolated from *Oenothera erythrosepala* (Hatano et al., 1990b) and *Cornus officinalis* (Hatano et al., 1989), respectively.

Corilagin **5** was produced from geraniin (isolated from *Geranium thunbergii*) (Okuda et al., 1982) in the following way. An aqueous solution (5 ml) of geraniin (100 mg) was treated with sodium dithionite (150 mg) at 70 °C for 1 h. Then, the solution containing 1-*O*-galloyl-2,4;3,6-bis-(*R*)-4,5,6,4,5,6-hexahydroxydiphenoyl-β-D-glucose was heated in a boiling-water bath for 2 h. The insoluble material (ellagic acid) was removed by centrifugation, and the supernatant was acidified with 1 M HCl. Then, the solution was passed through a SepPak C_{18} cartridge (Waters), and the adsorbed material was eluted with 20% MeOH, to give crude corilagin **5**. The crude corilagin **5** obtained from 500 mg of geraniin was further subjected to a Sephadex LH-20 chromatography (Pharmacia) with EtOH to give purified corilagin **5** (129 mg).

4.2. Precipitation tests

Mixtures of polyphenols (0.5, 0.25, and 0.125 mg/ml) and proteins (0.5 mg/ml) in phosphate buffers at pH 4 and 7 were placed in the wells of 96-well plates (Iwaki) for 2 days to see whether precipitates formed.

4.3. SEC analyses

The SEC analyses of solutions containing BSA were performed on an YMC Diol-300 column (8.0 mm i.d.×300 mm) in an oven set at 35 °C, using a KH_2PO_4–Na_2HPO_4 buffer (0.067 M) containing Na_2SO_4 (0.067 M) (pH 6.8) as the eluant at a flow rate 0.8 ml/min. An 8.0 mm i.d.×30-mm column with the same packing material was used as a guard column. UV Detection at 220 or 280 nm was effected on a Hitachi L-7455 photodiode-array detector. The calibration curve used to estimate molecular size was based on the retention behavior of thyroglobulin (MW 6.4×10^5), BSA (6.9×10^4), ovalbumin (4.5×10^4), myoglobin (1.8×10^4), and ribonuclease A (1.4×10^4) as standards. Since BSA showed a peak due to its dimer (1.38×10^5) on the chromatogram, this peak was also used as the standard. As shown in Fig. 5, the logarithms of the molecular weights and the retention volumes form linear relationship for the proteins larger than that of ovalbumin on the YMC column.

Each mixture of polyphenol (0.25 mg/ml) and protein (BSA or cytochrome *c*, 0.5 mg/ml) in 0.1 M KH_2PO_4–KH_2PO_4 buffer (pH 7) (0.5 ml) was kept at 35 °C in a water-bath for 24 h. When precipitates formed, the samples were injected onto the columns after centrifugation at 7000 *g* for 1 min.

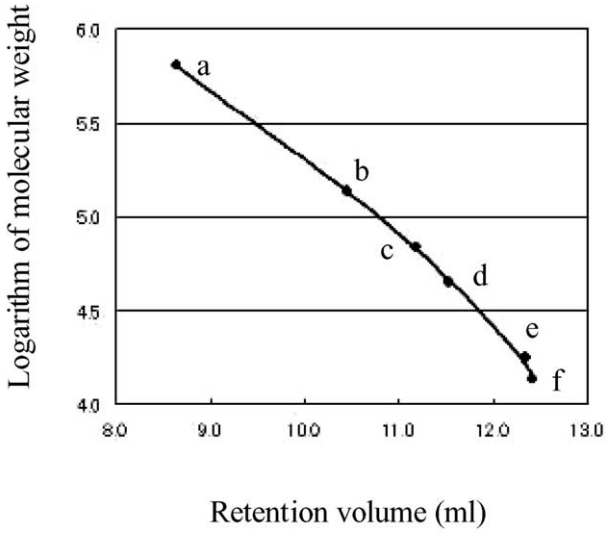


Fig. 5. Calibration curve indicating the relationship between the logarithms of the molecular weights and retention volumes of the proteins used as molecular weight standards on the YMC Diol-300 column: (a) thyroglobulin; (b) BSA dimer; (c) BSA monomer; (d) ovalbumin; (e) myoglobin; (f) ribonuclease A.

The M_n and M_w values were calculated according to the following equations:

$$M_n = \Sigma(H_i)/\Sigma(H_i/M_i)$$

$$M_w = \Sigma(H_iM_i)/\Sigma(H_i)$$

where H_i is the height of the point on the chromatographic profile from the base line at the retention volume i, and M_i is the molecular size calculated from the calibration line at the volume i.

For the analyses of solutions containing cytochrome c, a TOSOH Super SW2000 column (4.6 mm i.d.×300 mm) was used. TOSOH G4000SW_{XL} column (7.8 mm i.d.×300 mm) was also used for the analysis of the EGCG–BSA mixture.

4.4. Ultrafiltration of the mixture containing BSA–EGCG complex

The solution containing BSA and EGCG was kept at 35 °C for 24 h, and then subjected to ultrafiltration using a Millipore Ultrafree centrifugal tube equipped with a Biomax 100K NMWL membrane at 2100 g for 3 min. The resulting upper and lower phases were analyzed by SEC to compare the peak area due to the complex.

References

Baxter, N.J., Lilley, T.H., Haslam, E., Williamson, M.P., 1997. Multiple interaction between polyphenols and a salivary proline-rich protein repeat result in complexation and precipitation. Biochemistry 36, 5566–5577.

Charlton, A.J., Baxter, N.J., Khan, M.L., Moir, A.J.G., Haslam, E., Davies, A.P., Williamson, M.P., 2002. Polyphenol/peptide binding and precipitation. Journal of Agricultural and Food Chemistry 50, 1593–1601.

Hagerman, A.E., Butler, L.G., 1981. The specificity of proanthocyanidin-protein interactions. Journal of Biological Chemistry 256, 4494–4497.

Haslam, E., 1989. Plant Polyphenols: Vegetable Tannins Revisited. Cambridge University Press, Cambridge, pp. 154–219.

Haslam, E., 1998. Practical Polyphenolics: From Structure to Molecular Recognition and Physiological Action. Cambridge University Press, Cambridge, pp. 84–177.

Hatano, T., Ogawa, N., Kira, R., Yasuhara, T., Okuda, T., 1989. Tannins of Cornaceous plants. I. Cornusiins A, B and C, dimeric, monomeric and trimeric hydrolyzable tannins from *Cornus officinalis*, and orientation of valoneoyl group in related tannins. Chemical & Pharmaceutical Bulletin 37, 2083–2090.

Hatano, T., Hattori, S., Ikeda, Y., Shingu, T., Okuda, T., 1990a. Gallotannins having a 1,5-anhydro-D-glucitol core and some ellagitannins from Acer species. Chemical & Pharmaceutical Bulletin 38, 1902–1905.

Hatano, T., Yasuhara, T., Matsuda, M., Yazaki, K., Yoshida, T., Okuda, T., 1990b. Oenothein B, a dimeric, hydrolysable tannin with macrocyclic structure, and accompanying tannins from *Oenothera erythrosepala*. Journal of the Chemical Society, Perkin Transactions 1, 2735–2743.

Hatano, T., Hemingway, R.W., 1997. Conformational isomerism of phenolic procyanidins: preferred conformations in organic solvents and water. Journal of the Chemical Society, Perkin Transactions 2, 1035–1043.

Kawamoto, H., Nakatsubo, F., 1997. Solubility of protein complexed with galloylglucoses. Phytochemistry 46, 485–488.

Kohri, T., Matsumoto, N., Yamakawa, M., Suzuki, M., Nanjo, F., Hara, Y., Oku, N., 2001. Metabolic fate of (−)-[4-^{3}H]epigallocatechin gallate in rats after oral administration. Journal of Agriculture and Food Chemistry 49, 4102–4112.

Meng, X., Sang, S., Zhu, N., Lu, H., Sheng, S., Lee, M.-J., Ho, C.-T., Yang, C.S., 2002. Identification and characterization of methylated and ring-fission metabolites of tea catechins formed in humans, mice, and rats. Chemical Research in Toxicology 15, 1042–1050.

Nakagawa, K., Miyazawa, T., 1997. Chemiluminescence-high-performance liquid chromatographic determination of tea catechin, (−)-epigallocatechin 3-gallate, at picomole levels in rat and human plasma. Analytical Biochemistry 248, 41–49.

Okuda, T., Yoshida, T., Hatano, T., 1982. Constituents of *Geranium thunbergii* Sieb. et Zucc. Part 12. Hydrated stereostructure and equilibration of geraniin. Journal of the Chemical Society, Perkin Transactions 1, 9–12.

Okuda, T., Yoshida, T., Hatano, T., Mori, K., Fukuda, T., 1990. Fractionation of pharmacologically active plant polyphenols by centrifugal partition chromatography. Journal of Liquid Chromatography 13, 3637–3650.

Okuda, T., Yoshida, T., Hatano, T., 1995. Hydrolyzable tannins and related polyphenols. Fortschritte der Chemie organischer Naturstoffe 66, 1–117.

Richard, T., Verge, S., Berke, B., Vercauteren, J., Monti, J.-P., 2001. NMR and simulated annealing investigations of bradykinin in presence of polyphenols. Journal of Biomolecular Structure & Dynamics 18, 627–637.

Riedl, K.M., Hagerman, A.E., 2001. Tannin-protein complexes as radical scavengers and radical sinks. Journal of Agriculture and Food Chemistry 49, 4917–4923.

Scalbert, A., 1992. Quantitative methods for the estimation of tannins in plant tissues. In: Hemingway, R.W., Laks, P.E. (Eds.), Plant Polyphenols. Synthesis, Properties, Significance. Plenum Press, New York, pp. 259–280.

Shimizu, M., Shiota, S., Mizushima, T., Ito, H., Hatano, T., Yoshida, T., Tsuchiya, T., 2001. Marked potentiation of activity of β-lactams against methicillin-resistant *Staphylococcus aureus* by corilagin. Antimicrobial Agents and Chemotherapy 45, 3198–3201.

Takechi, M., Tanaka, Y., 1987. Binding of 1,2,3,4,6-pentagalloylglucose to proteins, lipids, nucleic acids and sugars. Phytochemistry 26, 95–97.

Unno, T., Kondo, K., Itakura, H., Takeo, T., 1996. Analysis of (−)-epigallocatechin gallate in human serum obtained after ingesting green tea. Bioscience, Biotechnology and Biochemistry 60, 2066–2068.

Yoshida, T., Hatano, T., Ito, H., Okuda, T., 2000. Chemical and biological perspectives of ellagitannin oligomers from medicinal plants. In: Atta-ur-Rahman (Ed.), Studies in Natural Products Chemistry, Vol. 23. Elsevier, Amsterdam, pp. 395–453.

Wroblewski, K., Muhandiram, R., Chakrabartty, A., Bennick, A., 2001. The molecular interaction of human salivary histatins with polyphenolic compounds. European Journal of Biochemistry 268, 4384–4397.

Zhu, M., Chen, Y., Li, R.C., 2000. Oral absorption and bioavailability of tea catechins. Planta Medica 66, 444–447.