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Development of Monoclonal Antibodies and Immunochromatographic Test Strips Applying Indirect Competitive Method for Daidzein Detection

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In this study, we aimed to detect daidzein secreted in the soybean rhizosphere using an indirect competitive immunochromatographic assay. A specific anti-daidzein monoclonal antibody was obtained through the establishment of a hybridoma. The antibody exhibited a detection limit of 5 ppb daidzein and showed strong cross-reactivity with daidzein, as determined by an indirect competitive method using a surface plasmon resonance sensor. Through the findings, a unique binding pattern differing from those of genistein and other saponins was observed. Enhanced sensitivity with the ability to detect daidzein concentrations as low as 10 nM (2.5 ng/mL) was achieved using immunochromatographic test strips with modified gold nanoparticles.

1. Introduction

Soybeans are an indispensable part of the Japanese diet, used for tofu, miso, soy sauce, and various other purposes such as fats, oils, and biodiesel fuels. However, Japan's self-sufficiency rate for soybeans was only 7% (25% for food use) as of 2009. This is because yields are unstable due to natural disasters such as heavy rainfall, typhoons, and cold weather, as well as soil problems. Soybeans secrete the isoflavone daidzein in the rhizosphere and symbiose with rhizobium bacteria to obtain the nitrogen necessary for growth. However, although it is known that daidzein is deeply involved in soybean growth, there is no established method for measuring plant metabolites in the rhizosphere during growth in the field without pulling out the target

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plants, and the details of rhizosphere daidzein and soybean growth have not been clarified.[1] In general, metabolites secreted from plant roots in hydroponics are measured by liquid chromatography–mass spectrometry analysis, but the extent of diffusion of secreted metabolites in the soil is not known. Therefore, if the daidzein concentration in the field can be easily quantified and this relationship clarified, it may help control soybean growth conditions and stabilize and improve soybean yields. If a stable method for measuring daidzein in the rhizosphere can be established, it could be applied to other plants and be useful for ensuring the stable supply of agricultural products in the future. We previously used the fluorescence of bovine serum albumin (BSA) to visualize isoflavones such as daidzein secreted from soybean roots and a surface plasmon resonance sensor using hetero-core fibers to detect plant-related compounds.[2–4] Other than pH meters, immunochromatography is the most used measurement method to detect chemical compounds in the field.[5–7] For plant-related substances, test strips for cadmium are commercially available, and other chemicals can also be rapidly evaluated.[8] Since daidzein is a low-molecular-weight compound, the sandwich method is difficult to apply, and the indirect competitive method is expected to be applied.[9–14] To obtain stable antibodies, it is desirable to obtain monoclonal antibodies. In the indirect competitive method, it is beneficial to have higher affinity between the antibody and the target than between the antibody and a similar substance to be immobilized on the sensor surface.[15]

In this study, we used a synthetic compound of a daidzein analog and *Concholepas concholepas* hemocyanin (CCH) as immunogens to obtain polyclonal antibodies in rabbits and monoclonal antibodies in rats. The sensitivity to daidzein of polyclonal antibodies, which can be acquired in a shorter period than monoclonal antibodies, was investigated and compared with that of monoclonal antibodies. The sensitivity of these antibodies to daidzein was evaluated using a surface plasmon resonance sensor. The cross-reactivity of the monoclonal antibodies was also evaluated. Since the rhizosphere of soybeans in the pod stage contains 1.7 ppm daidzein,[16] we aimed to measure the ppb level in consideration of dilution and at an earlier stage. An indirect competitive method was employed for immunochromatography using the rat monoclonal antibody, and a response curve was obtained by image analysis.

2. Materials and Methods

2.1 Materials

Daidzein ($C_{15}H_{10}O_4$, Mw 254.24), daidzin ($C_{21}H_{20}O_9$, Mw 416.38), genistein ($C_{15}H_{10}O_5$, Mw 270.24), and 7-*o*-carboxymethyl ether daidzein (CED, $C_{17}H_{12}O_6$, Mw 312.2) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Genistin ($C_{21}H_{20}O_{10}$, Mw 432.38) was obtained from Nagara Science (Gifu, Japan) and Saponin B was obtained from FUJIFILM Wako Pure Chemical Industries (Japan). A TNT solution in Milli-Q water was obtained from Chugoku Kayaku (Hiroshima, Japan). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (EDC) and *N*-hydroxysuccinimide (NHS) were obtained from Nacalai Tesque (Japan). Ovalbumin (OVA; albumin from chicken egg white), BSA, and CCH (Blue Carrier Immunogen) were obtained from Sigma-Aldrich, FUJIFILM Wako Pure Chemical Industries, and Thermo Scientific Pierce,

respectively. Carboxy-EG_6 undecanethiol was obtained from Dojin Chemical Laboratory, and mono-*N*-t-boc-NH-$dPEG_{11}$ amine and amino-$dPEG_4$-alcohol from Quanta BioDesign were used as reagents for the SPR sensor surface. Other reagents were from Kanto Chemical, FUJIFILM Wako Pure Chemicals, Tokyo Chemical Industry, and Sigma-Aldrich.

2.2 Production of immunogen and polyclonal and monoclonal antibodies

CED was prepared at a concentration of 2.0 mg/mL using *N,N*-dimethylformamide (DMF). NHS (4.6 mg) and sodium sulfate (101.2 mg) were added and the mixture was stirred. EDC (68.3 mg) was then added and the mixture was stirred for 7.5 h at room temperature. Then, 180 μL of the activated CED solution was slowly added in triplicate to CCH (50 mg) dissolved in 12.5 mM boric acid buffer (1 mL, pH 8.0). After reacting for 3 h, the reaction solution was dialyzed against Milli-Q water overnight and lyophilized to obtain CED-CCH. Solid-phase antigen for enzyme-linked immunosorbent assay (ELISA) was prepared in the same way using OVA.

Rabbit anti-CED-CCH polyclonal antibodies were prepared by Genenet (Fukuoka, Japan) according to a protocol involving five intradermal injections of immunogen into rabbits over 9 weeks. First, the immunogen prepared by the above procedure was added to Milli-Q water to make a solution with a concentration of 0.3 mg/mL (0.15 mg/mL only for the first time), which was emulsified with Freund's complete adjuvant. Immunogen solutions were injected intradermally into rabbits at weeks 0, 2, 4, 6, and 8. At weeks 5 and 7, blood was collected from the rabbit ears and used to measure antibody titers by ELISA. At week 9, whole blood was collected, and the antiserum was purified for IgG using Protein A. Finally, an anti-CED-CCH polyclonal antibody was obtained using 10 mM PB and 0.15 M NaCl (pH 7.2) as the solvent.

CED-CCH as the immunogen and CED-OVA for ELISA were sent to Hokudo (Sapporo, Japan) via Genenet (Fukuoka, Japan), and the establishment of hybridoma was commissioned. Four SD rats were immunosensitized. For the four immunized rats, antibody titers were measured by ELISA, and the two rats with superior antibody titers were used for cell fusion.

Myeloma cell line (SP2) and iliac lymph node B cells were fused by the polyethylene glycol (PEG) method. Screening was performed using the HAT medium, and positive hybridomas (20 strains) were selected on the basis of the absorbance of ELISA. The culture supernatant was filtered through a 0.2 μm filter and diluted fourfold with PBST (10 mM phosphate-buffered saline, 138 mM NaCl, 2.7 mM KCl, 0.05% Tween 20, pH 7.4). The binding ability of the antibodies in the culture supernatant was determined by an indirect competitive method using an SPR sensor. The binding rates of 50 ppb daidzein and 50 ppb CED were compared, and five hybridoma candidates were selected.

The five positive hybridoma strains were cloned by the limiting dilution method, and the culture supernatants were similarly compared by the indirect competitive method of the SPR sensor to determine the cells from which to collect ascites fluid. One of the cloned hybridoma strains was used to collect ascites fluid from three nude mice.

2.3 Fabrication of SPR sensor chip and evaluation of antibodies by indirect competitive method

SIA kit bare gold chips were sonicated in acetone for 10 min, ethanol for 2 min, and isopropanol for 2 min, followed by washing in SC1 solution (Milli-Q water: ammonia: hydrogen peroxide water 5:1:1) for 20 min at 90 ℃. The washed sensor gold chips were immersed in 10 mM carboxy-EG_6 undecanethiol in ethanol solution and placed in an incubator at 18.0 ℃. A self-assembled monolayer (SAM) was formed in 24 h. Then, the gold surface was rinsed with Milli-Q water (pumped three times with a pipette). The gold film was immersed in 0.4 M EDC in Milli-Q water solution and 0.1 M NHS in Milli-Q water at a ratio of 1:1, pumped three times with a pipette, and allowed to react at room temperature (25 ℃) for 30 min. The reaction was followed by rinsing with Milli-Q water. After carboxyl group activation, 10 mM mono-*N*-t-Boc-NH-$dPEG_{11}$ amine in Borate 8.5 buffer and amino-$dPEG_4$-alcohol (diluted in 10 mM Borate 8.5 buffer) were mixed dropwise at a ratio of 6:4 and pumped three times. The mixture was allowed to react for 60 min. After the reaction, the gold surface was rinsed with Milli-Q water. The Boc (tert-butoxycarbonyl) protecting group at the amino group end of the oligo ethylene glycol (OEG) chain was deprotected by immersing the gold surface in 4 M HCl for 1 h. Simultaneously with the hydrochloric immersion acid, 20 μl each of 0.4 mM EDC Milli-Q water solution, 0.1 mM NHS/DMF solution, and CED were placed in a microtube and allowed to react for 60 min to activate the analogs to be immobilized. After 60 min of reaction, the chip surface was rinsed with Milli-Q water, and 50 μl of the mixture of activated antigen analogs and 4 μl of triethylamine were added dropwise to immobilize the antigen analogs on the gold chip surface for 120 min.

The Biacore X100 Plus package (Cytiva) was used for SPR measurement. PBST was used as the running buffer. Only the antibody or antibody/daidzein mixtures were run for 5 min at 25 ℃ at a flow rate of 30 μL/min. After the distribution was finished, the flow was switched to a running buffer, and then the antibody was dissociated using 20 mM NaOH to regenerate the sensor surface. PBST was used to dilute the antibody solution and daidzein.

In the indirect competitive assay of the SPR sensor, four concentrations of antibody, 5 ppt, 50 ppt, 500 ppt, and 5 ppb, were measured for the rabbit-derived anti-daidzein polyclonal antibody at 50 ppm. For the rat anti-daidzein monoclonal antibody (diluted in PBST), the final concentration of the antibody was 6.25 ppm, and 5 ppt, 50 ppt, 500 ppt, 5 ppb, 50 ppb, and 500 ppb of daidzein were measured. The antibody and daidzein solutions were mixed at a 1:1 ratio. The SPR sensor response ($\Delta\theta_0$) during the flow of only the antibody was recorded, and $\Delta\theta_1$ was measured when a mixture of the antibody and daidzein or CED was introduced. The antibody binding rate was then calculated as $\Delta\theta_1/\Delta\theta_0$.[15] The calibration curve was obtained by four-parameter fitting using BIAevaluation software. The IC_{50} concentration is the concentration of analyte at which the binding is 50%. The cross-reactivity (CR) was calculated using IC_{50} as follows:[17]

$$CR = \left(\frac{C^*}{C} \right) \times 100, \tag{1}$$

where C^* is the concentration of the daidzein standard solution with 50% inhibition and C is the concentration of analyte that cross-reacts with 50% inhibition.

2.4 Immunochromatographic test strip fabrication and indirect competitive immunochromatography

The immunochromatography test strip based on the indirect competitive method for the detection of daidzein is shown in Fig. 1. The sample pad was a 10 mm × 30 mm Glass Fiber Diagnostic Pad (Merck GFDX103000). A drop of pretreatment solution [0.01 M PBS (2% BSA, 2.5% glycerol, 0.1% Tween20)] was added to the sample pad on a Teflon container and dried well in an incubator at 37 °C.

For gold-antibody conjugation, 400 µL of pH 7.1 PBS was mixed with 10 mL of 5 nm gold nanoparticles (AuNPs, Aldrich). Then 200 µL of 250, 375, or 875 µg/mL anti-daidzein antibody was added and the mixture was incubated for 30 min at room temperature. Next, 0.5 mL of 10% BSA was added and mixed well. The tubes were then centrifuged at 13,200 rpm for 60 min in 1.5 mL tubes. The supernatant was then discarded and resuspended in 100 µL of storage buffer [20 mM Tris (pH 8.0), 150 mM NaCl, 1% BSA (w/v)] and stored at 4 °C. The samples were diluted with 400 µL of storage buffer immediately before use.

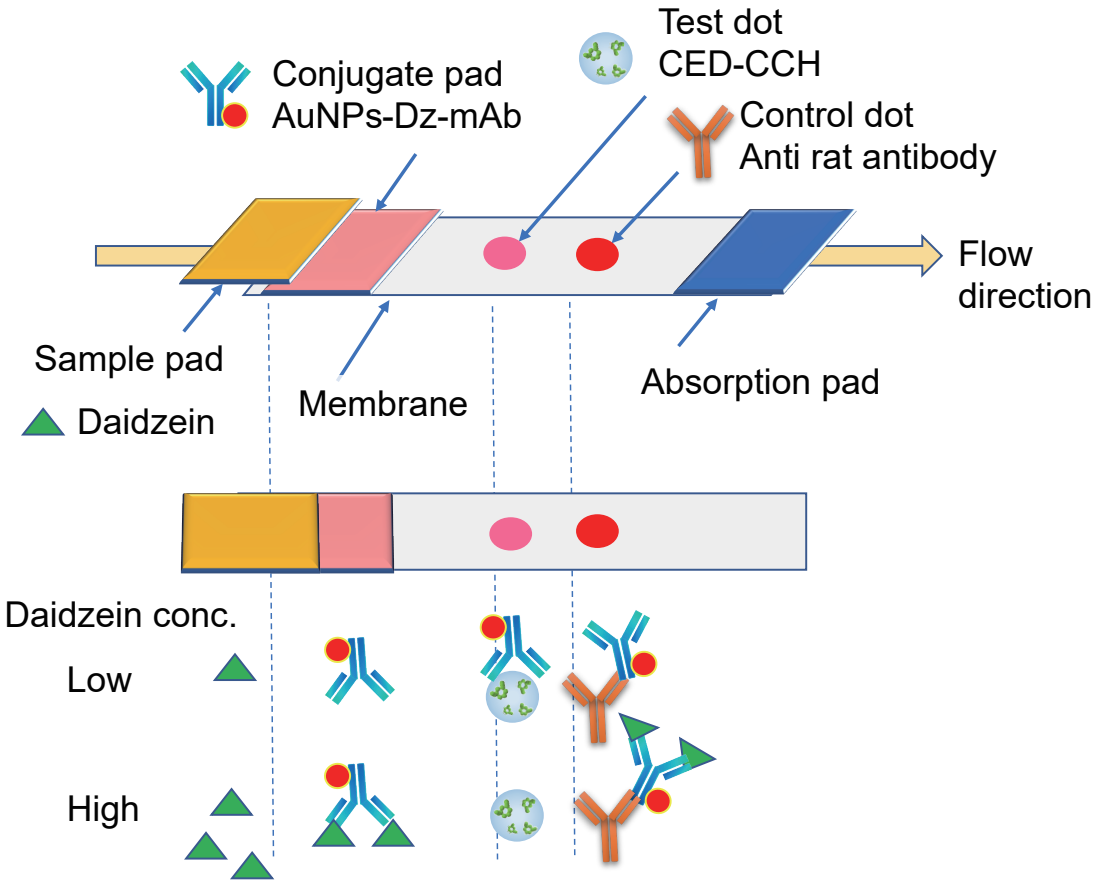

Fig. 1. (Color online) Diagram of immunochromatography test strip based on indirect competitive method for detection of daidzein.

The conjugate pad was also a 10 mm × 30 mm Glass Fiber Diagnostic Pad (Merck GFDX103000). AuNPs conjugated with the required concentration of anti-daidzein antibody were impregnated on glass microfiber sheets and dried on a Teflon container in a cool incubator at 37 °C, as for the sample pads.

Test dots and control dots were prepared instead of test lines and control lines as follows. [18] CED-CCH for test dots was prepared in the same way as the immunogen. The synthesized CED-CCH solution after dialysis without lyophilization was centrifuged, and the centrifugal supernatant was concentrated to around 4.0 mg/mL with an ultrafiltration filter (Amicon Ultra 0.5). The concentrated CED-CCH solution was spotted as test dots using a motorized micropipette.

The control dots were spotted with a stock solution (2.2 mg/mL) of rabbit anti-rat IgG (H&L) antibody (Rockland Immunochemicals, USA). A Whatman FF170HP membrane (20 mm × 50 m, Cytiva) was used as the membrane and a Whatman CF6 pad (22 mm × 50 m, Cytiva) was used as the absorption pad. The sample pad, conjugate pad, membrane, and absorbent pad were attached to a 0.2 mm (t) × 50 mm (L) polystyrene board with double-sided tape and cut into 5-mm-wide test strips.

For the indirect competitive immunochromatographic assay, 60 μL of a 0 or 10^{-2}–10^{4} nM daidzein solution diluted in Milli-Q water was added dropwise onto the sample pad, where 0 indicates a drop of Milli-Q water.

2.5 Image analysis of the test strips

Test strip images were acquired using an GT-X980 image scanner (Seiko Epson, Nagano, Japan) and iMeasure Scan Std Scanner-driven software (SW-IMS-S, iMeasure, Nagano, Japan). A Kodak Gray Scale test chart was read for calibration and ImageJ (Fiji) was used to obtain a profile of the test strip images. The test strips were scanned under the following conditions: reflection, 400 ppi resolution, 16-bit gray scale, densitometer mode checked, multiscan setting, and 16 additive averages, and saved as TIFF files. Optical density (O.D.) values were obtained by drawing a line through the center of the test dot and control dot in the immunochromatography scan image on ImageJ and plotting the O.D. values along the line on a plot profile. The O.D. values of the test dots were integrated from the profile, $\Delta\theta_0$ was defined as the time when Milli-Q was added dropwise, and $\Delta\theta_1$ was defined as the time when daidzein solution was added dropwise.

3. Results and Discussion

3.1 Screening of hybridoma

As shown in Fig. 2, CED is an analog of daidzein with a carboxyl group attached. This carboxyl group was used to bond to the amino group of CCH by the carbodiimide method.[19,20] Hybridomas were established by the rat lymph node method to obtain monoclonal antibodies using CED-CCH as an immunogen.[21,22] Five cell types with high absorbance in ELISA were

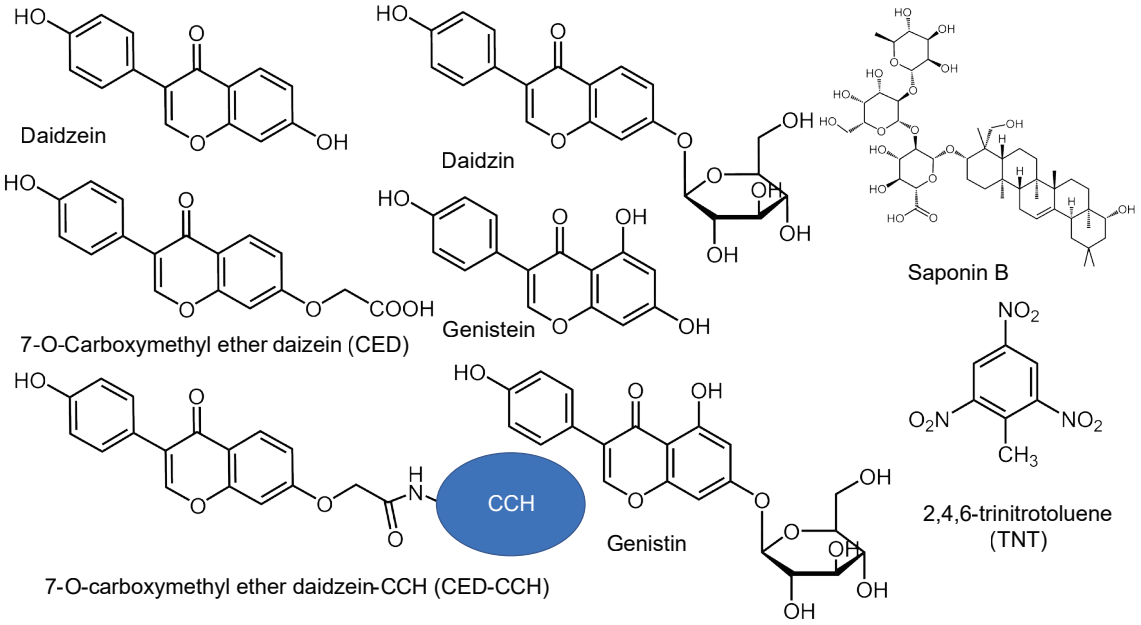

Fig. 2. (Color online) Chemical structures of daidzein and its analogs.

selected for screening after cell fusion. After the initial cloning for rat hybridoma establishment, screening by ELISA was conducted at Hokudo, the outsourcing company. Subsequently, we evaluated the culture supernatants from wells with high absorbance using the indirect competitive method with the SPR sensor. The sensor surface was prepared by modifying the SAM reagent using a previously reported method.[23] The results of evaluating 10 candidate hybridomas are shown in Table 1. The bound percentages of 5H12(E2) and 5H12(H3) were decreased by 50 ppb CED but not by 50 ppb daidzein. 1A12(C1), 1A12(F1), 2C2(E1), 2C2(G5), 3D5(D1), 3D5(E2), 5E8(B3), and 5E8(B11) exhibited a decreased bound percentage with both daidzein and CED, indicating that the antibodies in these culture supernatants bind to both substances. Notably, 5E8(B3) and 5E8(B11) demonstrated reduced binding to the sensor surface when mixed with daidzein compared with their binding with CED. This difference was further emphasized by a lower binding rate, as evidenced by the ratio with $\Delta\theta_0$. This means that the antibodies in these culture supernatants bind more strongly to daidzein than to CED. Among the 10 candidate hybridomas, 1A12(F1), 2C2(E1), 3D5(D1), 5E8(B3), and 5E8(B11) were selected for final cloning.

These culture supernatants were evaluated by the indirect competitive method using the SPR sensor, and 5E8(B3) and 5E8(B11) showed lower bound percentages to daidzein than CED. CED is used as a hapten of the immunogen and is immobilized on the sensor surface. In the indirect competitive method, higher sensitivity in measurement can be achieved when the affinity between the immobilized analog on the sensor surface and the antibody is lower than that between the antibody and the target substance.[15] In addition, the binding rate of 5E8(B3) was higher than that of 5E8(B11) when the amounts of binding were compared; therefore, 5E8(B3) was chosen for ascites amplification. The hybridoma is registered as QM2023-0060 with the Material Management Center (MMC).

Table 1
Response of culture supernatants to daidzein and CED by indirect competitive method using SPR sensor.

Culture supernatant (Well No.)	Analyte	Bound (%)				
		1st	2nd	3rd	Mean	SD
1A12(C1)	Daidzein	41.6	48.1	48.5	46.1	3.2
	CED	33.7	41.1	41.2	38.7	3.5
1A12(F1)	Daidzein	52.6	62.0	65.0	59.9	5.3
	CED	44.0	54.9	58.5	52.5	6.2
2C2(E1)	Daidzein	84.6	86.6	86.2	85.8	0.9
	CED	76.5	78.6	77.9	77.7	0.9
2C2(G5)	Daidzein	90.8	90.6	90.1	90.5	0.3
	CED	84.6	83.8	83.2	83.9	0.6
3D5(D1)	Daidzein	92.2	90.7	90.1	91.0	0.9
	CED	87.5	85.9	84.9	86.1	1.1
3D5(E2)	Daidzein	86.1	85.3	85.2	85.5	0.4
	CED	77.0	75.4	74.8	75.7	0.9
5E8(B3)	Daidzein	58.2	54.7	52.8	55.2	2.2
	CED	74.6	72.7	71.7	73.0	1.2
5E8(B11)	Daidzein	75.9	72.0	69.8	72.6	2.5
	CED	81.2	81.7	80.9	81.3	0.3
5H12(E2)	Daidzein	96.3	103.1	104.5	101.3	3.6
	CED	82.0	85.1	86.3	84.5	1.8
5H12(H3)	Daidzein	91.0	102.3	103.0	98.8	5.5
	CED	85.4	82.9	79.7	82.7	2.3

3.2 Evaluation of antibodies using SPR sensor

Figure 3 shows the response characteristics of rabbit polyclonal antibodies and rat monoclonal antibodies obtained from CED-CCH immunogen by the indirect competitive method using a surface plasmon resonance sensor. The anti-CED-CCH polyclonal antibody (a-CED-CCH pAb) binding decreased at a daidzein concentration of 50 ppt but stabilized at 500 and 5000 ppt with a bound percentage level of about 83%. This may be due to the polyclonal antibody binding to the CED immobilized on the sensor surface and the small percentage of antibody binding to daidzein. The assay's detection limit was determined to be 10 ppt, three times higher than the standard deviation of 0.8 at a concentration of 5 ppt. Although daidzein was detectable, precise quantification was not attainable.

The binding of the anti-daidzein rat monoclonal antibody (a-Dz mAb) began to decrease at 5000 ppt (5 ppb). At 500000 ppt (500 ppb), the bound percentage was 8.2%, which decreased with increasing daidzein concentration. The detection limit was calculated to be 5 ppb with a standard deviation of three times the concentration at 50 ppt.

The CR of a-Dz mAb against daidzein-related compounds was examined. a-Dz mAb was cross-reactive to daidzein-related compounds, and the results are summarized in Table 2. As shown in Fig. 1, daidzin is a glycoside of daidzein and has the same structure except for the sugar, indicating that the antibody recognizes the structure of daidzein. For CED, the CR was 33.1%, a large difference from daidzein, because cells were selected by comparing the binding to daidzein and to CED during hybridoma selection. Genistein and genistin, a glycoside of

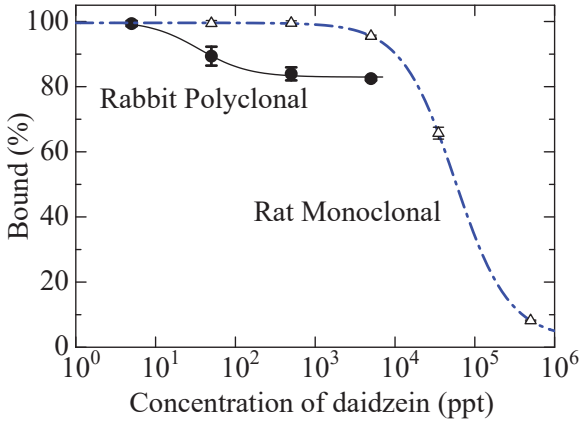

Fig. 3. (Color online) Response curves obtained by indirect competitive method for detection of daidzein using a-CED-CCH pAb and a-Dz mAb.

Table 2
IC50 and cross-reactivities of anti-daidzein monoclonal antibody.

Analyte	IC_{50} (p mol)	Cross-reactivity (%) (molar)
Daidzein	7.4	100.0
Daidzin	6.0	123.1
CED	22.4	33.1
Genistein	33.8	21.9
Genistin	32.9	22.5
Saponin B	—	—
Trinitrotoluene (TNT)	—	—

genistein, have one more hydroxyl group than daidzein, but both have a CR of about 22%. Therefore, this antibody is considered to recognize the presence or absence of hydroxyl groups. Saponin B was chosen because it is a plant secondary metabolite secreted from soybean roots, and TNT was selected due to its low molecular weight and a benzene ring, albeit with a completely different structure. The a-Dz mAb did not exhibit binding to these molecules.

Figure 4 shows scans of test strips prepared with 5 nm AuNPs, 250 μg/mL Ab, and drops of 0 or 1×10^{-2}–1×10^{4} M daidzein. Both test and control dots appear to be colored. However, the test dots are lighter in color than the control dots. The test dots gradually become lighter as the daidzein concentration increases and are much lighter at 1×10^{4}. Test strips of 5 nm AuNPs conjugated with 250, 375, and 875 μg/mL antibody solutions were tested three times for each concentration of daidzein. The results are shown in Fig. 5. The IC_{50} values of 250, 375, and 875 μg/mL Ab were 3, 19, and 62 nM, respectively, indicating that the AuNPs modified with 250 μg/mL Ab were the most sensitive. As the amount of antibody bound to a single AuNP increases, the amount of daidzein required to inhibit the binding of the antibody to the CED-CCH of the test dot increases. Thus, in the 250, 375, and 875 μg/mL antibody solutions, the amount of antibody bound to a single AuNP increased with the antibody concentration.

However, the error bars were too large to obtain quantitative results. The test dots were spotted manually, which may have been one of the reasons for the large error bars. At present,

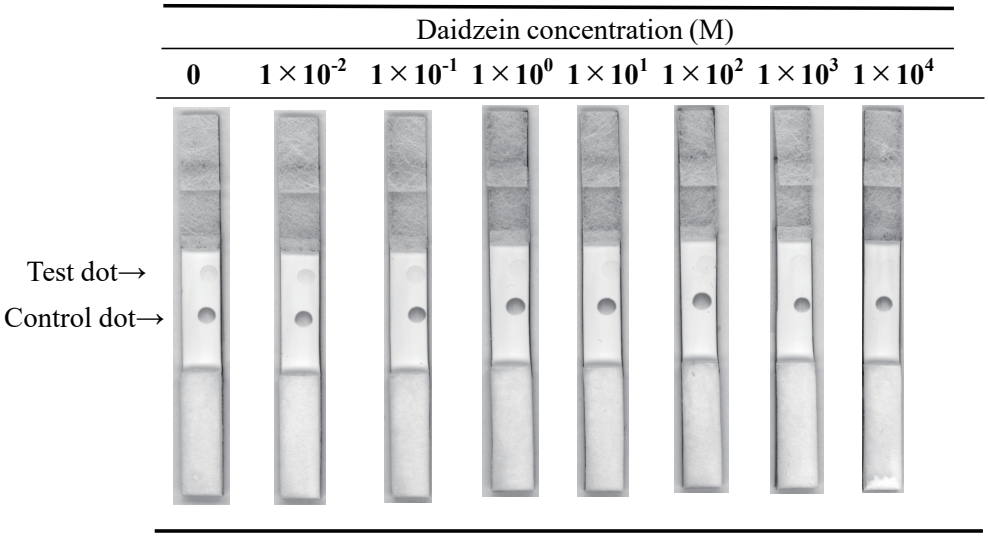

Fig. 4. Scanned images of immunochromatographic test strips prepared with 5 nm AuNPs and 250 μg/mL Ab after dropwise addition of 0 or 1×10^{-2}–1×10^{4} M daidzein.

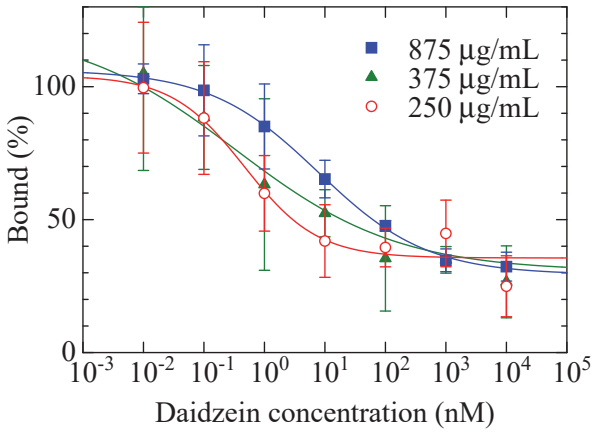

Fig. 5. (Color online) Daidzein response characteristics of indirect competitive immunochromatography at antibody concentrations of 250, 375, and 875 μg/mL when conjugated with AuNPs.

10^{1} nM (2.5 ng/mL) can be detected using 250 μg/mL Ab, as judged by visual inspection. For quantitative measurement in the future, it is necessary to reduce the error bars and to consider the sampling method.

4. Conclusions

In this study, immunochromatography was developed for the periodic measurement of the daidzein concentration in the soybean rhizosphere. CED, an analog of daidzein, and CCH, a protein, were synthesized and used as immunogens. Rabbit polyclonal antibody and rat

monoclonal antibody were produced using CED-CCH. A 10 ppt limit of detection of daidzein was obtained with the rabbit polyclonal antibody by the indirect competitive method using an SPR sensor. However, the daidzein concentration was not quantifiable. The rat monoclonal antibody showed a concentration-dependent response to daidzein with a 5 ppb limit of detection, which was higher than that of the rabbit polyclonal antibody. The CR of the rat monoclonal antibody was low except for that of daidzin, a glycoside of daidzein, and high selectivity was obtained.

To detect daidzein, immunochromatographic test strips were developed using an indirect competitive method. Although a response curve was obtained by image analysis, the error bars were large and quantitative results were not obtained. However, 10 nM (2.5 ng/mL) daidzein was detectable. Thus, the immunochromatographic test strip capable of detecting daidzein employing the indirect competitive method was realized.

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References

1 A. Sugiyama: Biosci., Biotechnol., Biochem. **85** (2021) 1919. https://doi.org/10.1093/bbb/zbab106
2 A. Hosoki, M. Nishiyama, N. Kumekawa, K. Watanabe, R. Yatabe, Y. Tahara, T. Onodera, A. Sugiyama, and N. Sakurai: Opt. Commun. **524** (2022) 128751. https://doi.org/10.1016/j.optcom.2022.128751
3 A. Hosoki, M. Nishiyama, N. Kumekawa, K. Watanabe, R. Yatabe, Y. Tahara, T. Onodera, A. Sugiyama, and N. Sakurai: Opt. Laser Technol. **169** (2024) 110045. https://doi.org/10.1016/j.optlastec.2023.110045
4 T. Onodera, H. Miyazaki, X. Li, J. Wang, M. Nakayasu, R. Yatabe, Y. Tahara, A. Hosoki, N. Sakurai, and A. Sugiyama: Biosens. Bioelectron. **196** (2022) 113705. https://doi.org/10.1016/j.bios.2021.113705
5 M. Yamaguchi, S. Kosaka, Y. Tahara, and A. Date: ECS Trans. **16** (2008) 67. http://dx.doi.org/10.1149/1.2981106
6 Y. Tahara, S. Yoshikawa, V. Shetty, and M. Yamaguchi: Sens. Mater. **22** (2010) 427. http://dx.doi.org/
7 X. Yan and K. C. Persaud: IEEE Sens. J. **19** (2019) 404. http://dx.doi.org/10.1109/JSEN.2018.2878449
8 K. Abe, Y. Sakurai, A. Okuyama, K. Sasaki, and K. Tawarada: J. Sci. Food Agric. **89** (2009) 1097. https://doi.org/10.1002/jsfa.3541
9 W. Leung, P. Chan, F. Bosgoed, K. Lehmann, I. Renneberg, M. Lehmann, and R. Renneberg: J. Immunol. Methods **281** (2003) 109. https://doi.org/10.1016/j.jim.2003.07.009
10 N. A. Byzova, E. A. Zvereva, A. V. Zherdev, S. A. Eremin, and B. B. Dzantiev: Talanta **81** (2010) 843. https://doi.org/10.1016/j.talanta.2010.01.025
11 N. A. Byzova, E. A. Zvereva, A. V. Zherdev, S. A. Eremin, P. G. Sveshnikov, and B. B. Dzantiev: Anal. Chim. Acta **701** (2011) 209. https://doi.org/10.1016/j.aca.2011.06.001
12 A. Apilux, S. Rengpipat, W. Suwanjang, and O. Chailapakul: Excli J. **17** (2018) 1198. http://dx.doi.org/10.17179/excli2018-1824
13 L. Guo, X. Wu, L. Liu, H. Kuang, and C. Xu: Small **14** (2018) 1701782. https://doi.org/10.1002/smll.201701782
14 S. Sakamoto, G. Yusakul, P. Nuntawong, T. Kitisripanya, W. Putalun, T. Miyamoto, H. Tanaka, and S. Morimoto: J. Chromatogr. B **1048** (2017) 150. https://doi.org/10.1016/j.jchromb.2017.01.032
15 T. Onodera and K. Toko: Sensors (Basel) **14** (2014) 16586. http://dx.doi.org/10.3390/s140916586
16 Z.-Y. Guo, C.-H. Kong, J.-G. Wang, and Y.-F. Wang: Soil Biol. Biochem. **43** (2011) 2257. http://dx.doi.org/10.1016/j.soilbio.2011.07.022
17 A. Zeck, M. G. Weller, and R. Niessner: Fresenius J. Anal. Chem. **364** (1999) 113. http://dx.doi.org/doi.org/10.1007/s002160051309
18 Z. Bai, Y. Luo, W. Xu, H. Gao, P. Han, T. Liu, H. Wang, A. Chen, and K. Huang: J. Sci. Food Agric. **93** (2013) 3743. https://doi.org/10.1002/jsfa.6232
19 G. T. Hermanson: Bioconjugate Techniques (Academic Press, Massachusetts, 2008) https://doi.org/10.1016/B978-0-12-370501-3.X0001-X

20 S. Nakamura, R. Yatabe, T. Onodera, and K. Toko: Biosensors **3** (2013) 374. http://dx.doi.org/doi:10.3390/bios3040374
21 Y. Sado, M. Kagawa, Y. Kishiro, K. Sugihara, I. Naito, J. M. Seyer, M. Sugimoto, T. Oohashi, and Y. Ninomiya: Histochem. Cell Biol. **104** (1995) 267. http://dx.doi.org/10.1007/BF01464322
22 Y. Tanaka, R. Yatabe, K. Nagatomo, T. Onodera, K. Matsumoto, and K. Toko: IEEE Sens. J. **13** (2013) 4452. https://doi.org/10.1109/JSEN.2013.2269697
23 Y. Mizuta, T. Onodera, P. Singh, K. Matsumoto, N. Miura, and K. Toko: Biosens. Bioelectron. **24** (2008) 191. http://dx.doi.org/10.1016/j.bios.2008.03.042