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Comparison of Properties of Polyclonal Anti-*N*-Acetylchitooligosaccharides and Anti-Chitooligosaccharides Antibodies Produced for ELISA

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Abstract To develop the enzyme-linked immunosorbent assay (ELISA) for the analysis of *N*-acetylchitooligosaccharides (NACOS) and chitooligosaccharides (COS), specific antibodies (Abs) were produced, and their properties were compared. *N*-acetylchitohexaose (NACOS6), chitohexaose (COS6), and COS mixture (COSM) conjugated to bovine serum albumin (BSA) were used to immunize rabbits. By the use of specific Abs and NACOS6-horseradish peroxidase (HRP), COS6-HRP, and COSM-HRP conjugates, competitive direct ELISA (cdELISA) was developed. The detection limits of NACOS6 by the anti-NACOS6 Ab and COS6 by the anti-COS6 and the anti-COSM Abs in the cdELISAs were about 0.2, 2, and 2 ng/ml (ppb), respectively. In the cdELISA, the anti-NACOS6 Ab was found to recognize NACOS3-NACOS6, but not *N*-acetyl-D-glucosamine (GlcNAc), NACOS2, and COSs; the anti-COS6 Ab recognized COS2-COS6 and COSM, but not glucosamine (GlcN) and NACOSs. The recognition pattern of the anti-COSM Ab was almost the same as that of the anti-COS6 Ab, except that the former recognized COS2 and COS3 slightly better than the latter.

Key words: *N*-acetylchitooligosaccharides (NACOS), chitooligosaccharides (COS), polyclonal antibody, ELISA, cross-reactivity

Chitin, muco-polysaccharide, consists of β-1,4-glycoside linkages of *N*-acetyl-D-glucosamine (GlcNAc) and is barely soluble in general solvents, because of the rigid crystalline structure through intra and inter residual hydrogen bonds between acetamide and hydroxyl groups on GlcNAc residues [9]. Chitosan, one of the chemical derivatives of chitin, however, is quite soluble in water [4].

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Chitooligosaccharides (COSs) are oligomers of glucosamine (GlcN), and *N*-acetylchitooligosaccharides (NACOSs) are oligomers of GlcNAc [17]. COSs and NACOSs have recently received growing attention, because they have a variety of biological activities, such as inhibitory effects on the growth of bacteria and fungi [15], antihypertension, hypocholesterolemic and antihyperlipemic activity, antitumor [5] and immunostimulating activity [14], and eliciting pathogenesis-related proteins in higher plants [6, 7]. Chain length and degree of deacetylation (DDA) of COSs are considered as the most important factors to influence biological activities: COSs, longer than a hexamer in chain length, have stronger antimicrobial, antitumor, and immunopotentiating activities than the shorter oligosaccharides, even though the shorter oligosaccharides have also significant activities [13]. Suzuki *et al* [18] and Tokoro *et al.* [19] found that chitohexaose (COS6) and *N*-acetylchitohexaose (NACOS6) suppressed sarcoma-160 and meth-A tumor growth in mice, respectively, and NACOS6 was also found to have antitumor activity against Lewis lung carcinoma and some immunostimulating activities [18].

In order to detect NACOSs and COSs, conventional methods such as HPLC, TLC, and colorimetric method have been used [16, 20,]. However, they are not satisfactory for the detection of NACOSs and COSs in multi-component mixtures such as foods. For TLC [21] and HPLC analyses [20], it is necessary to extract and extensively purify COSs. In the colorimetric method of Rondle and Morgan [16], contaminating detrimental materials in the sample can interfere with the assay, and the reactivity of the reagent toward COSs is not dependent on the degree of polymerization (d.p.) of COSs; that is, the reagent has the highest reactivity to GlcN, but lower reactivity to higher d.p. of COSs. Generally, an enzyme-linked immunosorbent assay (ELISA) provides highly sensitive, rapid, and precise means to estimate biological

parameters, with the added advantage that they can rapidly analyze a large number of samples [10, 11]. Kim *et al.* [8] produced an anti-COS mixture (COSM) polyclonal antibody specific to COSM, and established competitive direct ELISA (cdELISA) to detect COSs. However, it was not possible to detect NACOSs by the cdELISA, using the anti-COSM Ab.

In this research, to develop cdELISAs for the detection of NACOSs and/or COSs in complex matrices such as foods, three different antibodies (Abs), anti-NACOS6, anti-COS6, and anti-COSM Abs, were raised in rabbits, and their characteristics were compared.

MATERIALS AND METHODS

Materials

D-Glucosamine (GlcN), 1-ethyl-3,3'-dimethylamino-propyl-carbodiimide (EDPC), 1,1'-carbonyldiimidazole (CDI), 3,3',5,5'-tetramethyl-benzidine (TMB), bovine serum albumin (BSA, Cat#A7906), horseradish peroxidase (HRP, Cat#P8125), Tween 20, Freund's complete adjuvant, Freund's incomplete adjuvant, and goat anti-rabbit IgG-peroxidase conjugate (Cat#A6154) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Anhydrous methyl sulfoxide was obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Chitobiose (COS2), chitotriose (COS3), chitotetraose (COS4), chitopentaose (COS5), chitohexaose (COS6), *N*-acetyl-D-glucosamine (GlcNAc), *N*-acetylchitobiose (NACOS2), *N*-acetylchitotriose (NACOS3), *N*-acetylchitotetraose (NACOS4), *N*-acetylchitopentaose (NACOS5), and NACOS6 were purchased from Seikagaku Co. (Tokyo, Japan). COSM, which is composed of 3.6% COS2, 20.0% COS3, 23.9% COS4, 25.8% COS5, 17.3% COS6, and 9.3% COS7, was purchased from Amicogen Co. (Jinju, Korea).

New Zealand White rabbits were purchased from Hallim Laboratory Animal Co. (Hwasung, Korea). Microtiter plates, Maxisorp™ from Nunc Co. (Cat#446612, Roskilde, Denmark), and a microplate reader, THERMOmax™ from Molecular Devices Co. (Sunnyvale, CA, U.S.A.) were used. All chemicals were of GR grade or better.

Preparation of Immunogen

Conjugate of NACOS6 (M.W., 1,237 Da; DDA, 0%) and BSA for an immunogen was prepared according to the method of Hearn [3] and Bartling *et al.* [2]. Briefly, 25 mg of NACOS6 and 25 mg of CDI were added to 150 μl of anhydrous methyl sulfoxide, and the mixture was stirred at room temperature (R.T.) for 2 h. One-hundred μl of BSA (20 mg/ml in 0.01 M Na-carbonate buffer, pH 9.0) were added to the mixture and stirred for 24 h at 4°C. The mixture was purified through a Sephadex G-25 (25×1.5 cm) column, and the fraction was spectrophotometrically monitored at 280 nm. The fractions containing NACOS6-BSA conjugate were pooled and lyophilized.

COS6 (M.W., 1,204 Da; DDA, 100%) or COSM (average M.W. estimated on the basis of COS distrubution, 909 Da; DDA, 97%) was coupled to BSA by the modified method of Lönngren *et al.* [12] and Kim *et al.* [8]. In the coupling with EDPC, the carboxyl group of BSA was activated to *O*-acylisourea and coupled with the amine group of GlcN unit. Briefly, 650 mg of COS6 or COSM were added to a solution of BSA [200 mg in 2.0 ml of double distilled water (DDW)]. The pH was adjusted to 4.75 with 1.0 N HCl. A solution of EDPC (315 mg in 1.0 ml of DDW) was added dropwise over 30 min at R.T., while the pH was maintained at 4.75 by the addition of 0.5 N HCl. These solutions were kept for 6 h at R.T., and the pH was adjusted again. After an additional 72 h stirring, the modified protein suspensions were dialyzed against DDW and then lyophilized.

Production of NACOS6-BSA, COS6-BSA, and COSM-BSA conjugates was confirmed by SDS-PAGE according to the method of Bollag *et al.* [1].

Preparation of Horseradish Peroxidase (HRP) Conjugate

NACOS6-HRP conjugate was prepared as follows: Twenty-five mg of NACOS6 and 25 mg of CDI were added to 150 μl of anhydrous methyl sulfoxide, and the mixture was stirred for 2 h at R.T. Twenty-five μl of NACOS6 solution were added to 1 ml of HRP solution (50 mg/ml in 0.01 M Na-carbonate buffer, pH 9.0), and the reaction mixture was stirred at 4°C for 24 h. The mixture was dialyzed 4 times against PBS. The solution was purified with Sephadex G-25 (25×1.5 cm) column, and the fraction was spectrophotometrically monitored at 280 nm. The fractions containing NACOS6-HRP conjugate were stored at 4°C for later use.

COS6-HRP and COSM-HRP conjugates for cdELISA were prepared as follows: Periodate-activated HRP was first prepared. Twenty mg of HRP in 10 ml of DDW and 21.4 mg of $NaIO_4$ in 1 ml DDW were mixed and stirred for 10 min at R.T. The mixture was dialyzed against 5 mM Na-acetate buffer, pH 4.0. The pH of activated HRP was adjusted to 9.0 with 0.2 M Na-carbonate buffer, pH 9.5. One half mg each of COS6 or COSM was added to 1.0 ml each of the activated HRP and they were stirred at R.T. for 2 h. A 100 μl reduction solution (0.1 M $NaBH_4$) was added to each reaction mixture and kept for 2 h at 4°C. Thereafter, the purification procedure was the same as described above.

Production of Antisera

Three rabbits were subcutaneously immunized with emulsions of NACOS6-BSA, COS6-BSA, or COSM-BSA conjugate, respectively. Immunizations were carried out as follows: the concentration of immunogen was adjusted to

1 mg/ml. For the first injection, the portions were emulsified with Freund's complete adjuvant (1:1) using a Luer Lock syringe, and 1 ml of emulsified immunogen (0.5 mg/head) was immunized in the rear footpads of the rabbits. In the following injections, the portions were emulsified with Freund's incomplete adjuvant and injected in the back of the rabbits. Bleeding on the vein of the rabbits' ears was performed one week after each injection. The blood was kept for 2 h at R.T. and centrifuged at 3,000 ×*g* for 20 min. Antisera were isolated from the blood, added with NaN_3 to a final concentration of 0.02%, and stored at -70°C until use.

ELISA

In non-competitive ELISA, each well of polystyrene microtiter plate was coated with 100 µl of NACOS6-BSA conjugate (2 µg/ml) in a coating buffer (0.02 M Tris, 0.15 M NaCl buffer, pH 9.0). Antigen-treated plates were stored overnight at 4°C, and each well was washed three times with 150 µl of a washing buffer (0.02 M Tris, 0.15 M NaCl buffer, pH 7.4, with 0.02% Tween 20). To each well, 100 µl of the anti-NACOS6-BSA antiserum, which was appropriately diluted with the washing buffer containing 1% BSA, was added and left for 1 h. After washing three times, 100 µl of goat anti-rabbit IgG-HRP conjugate as a secondary Ab diluted to 1/5,000 in the washing buffer was added to each well and left for 1 h. After washing three times, 100 µl of a substrate solution (0.01% TMB, 0.05 M phosphate citrate buffer, pH 5, 0.002% H_2O_2) was added, developed for 30 min at R.T., and the reaction was stopped with 50 µl of 2 M H_2SO_4. Absorbance was measured at 450 nm with a microplate reader.

In competitive indirect ELISA (ciELISA), the procedure was the same as in the non-competitive ELISA, except that 100 µl of NACOS6 solution and anti-NACOS6 antiserum diluted to 1/10,000 in the washing buffer containing 1% BSA (1:1 mixture) were added to each well of the NACOS6-BSA conjugate-coated plate in place of the antiserum used in the experiment of the non-competitive ELISA.

In competitive direct ELISA (cdELISA), each well of the plates was coated with 100 µl of protein A/G (2 µg/ml, in the coating buffer) overnight at 4°C. After washing three times, each well was treated with 100 µl of the anti-NACOS6 Ab (antiserum diluted 1/5,000 in the washing buffer) for 1 h at R.T. After washing three times, 100 µl of NACOS6 standard or sample and NACOS6-HRP conjugate (1:1 mixture) were added into each well and left for 1 h at R.T. After washing three times, the coloring reaction was the same as described for the non-competitive ELISA. The ELISA procedure of the anti-COS6 and the anti-COSM Ab was performed according to the same method as described for anti-NACOS6 Ab, except that the COS6-HRP or COSM-HRP conjugate was used instead of the NACOS6-HRP conjugate.

Cross-Reactivities

Cross-reactivities of the anti-NACOS6, anti-COS6, and anti-COSM Abs toward different d.p. of COSs, NACOSs, and COSM were determined for cdELISA, as below.

$$\text{Cross-reactivity (\%)} = \frac{\text{Concentration of free hapten displacing 50\% of hapten-HRP}}{\text{Concentration of oligosaccharides displacing 50\% of hapten-HRP}} \times 100$$

RESULTS

Antibody Production

The NACOSs and COSs were not quite immunogenic, therefore, they had to be coupled with carrier proteins for Ab production using NACOS6, COS6, and COSM as haptens in immunogen preparation and competitors in the competitive ELISA; each hapten was coupled as described in Materials and Methods. The conjugates were confirmed by SDS-PAGE. As seen in Fig. 1, the NACOS6-BSA band became broader and shifted to higher than 66 kDa BSA after conjugation, indicating that NACOS6 molecules were covalently well bound to BSA. After conjugation, the increased molecular weight of BSA represented linkage of each hapten to BSA, however, heterogeneity of the

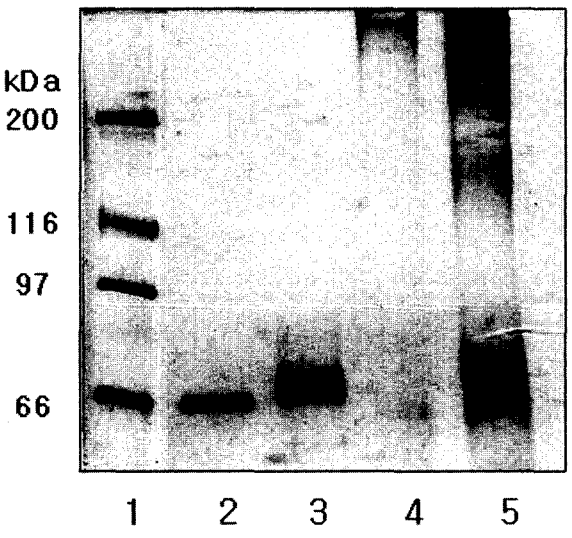

Fig. 1. SDS-PAGE patterns of NACOS6-BSA, COSM-BSA, and COS6-BSA conjugates that were used for the production of anti-NACOS6, anti-COSM, and anti-COS6 Abs.
Electrophoresis was done on 8% polyacrylamide gel (8%) at pH 8.8 in Tris buffer. Lane 1, Molecular weight marker (kDa); lane 2, Bovine serum albumin; lane 3, NACOS6-BSA conjugate; lane 4, COSM-BSA conjugate; lane 5, COS6-BSA conjugate.

coupled haptens did not show a clear-cut band. Also, some aggregates of BSA molecules appeared during conjugation of COS6-BSA and COSM-BSA, because molecular weight bands larger than the 132 kDa BSA dimer were seen in Fig. 1.

In spite of the SDS-PAGE patterns, specific antibodies were induced by immunization of the conjugates into rabbits. It was confirmed by non-competitive ELISA that each antiserum contained a high titer of polyclonal antibodies against each hapten (data not shown).

Competitive Direct ELISA (cdELISA)

By the use of each specific Ab and NACOS6-HRP, COS6-HRP, and COSM-HRP conjugates, cdELISAs were established. In the cdELISAs, the detection limit of NACOS6 by the anti-NACOS6 Ab and those of COS6 by the anti-COS6 and anti-COSM Abs were about 0.2, 2, and 2 ng/ml (ppb), respectively (Fig. 2).

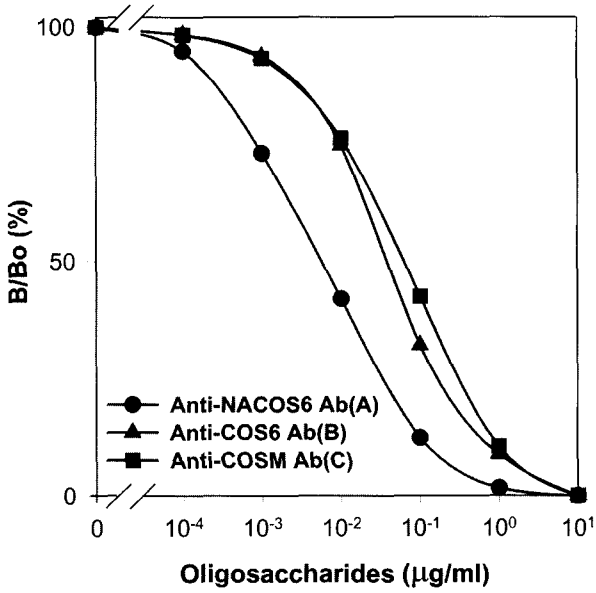

Fig. 2. Standard curves for oligosaccharides (COS6 and NACOS6), as determined by cdELISA.
B/Bo means relative binding of NACOS6-HRP, COS6-HRP, and COSM-HRP conjugates to each specific Ab in the presence of oligosaccharides. (A) Each well of the plate was coated with 100 μl of 2 μg/ml protein A/G in the coating buffer overnight at 4°C. After washing three times, each well was treated with 100 μl of the anti-NACOS6 Ab (antiserum diluted 1/5,000 in the washing buffer) for 1 h at R.T. After washing, 100 μl of 1:1 mixture of NACOS6: NACOS6-HRP conjugate (diluted 1/3,000 in the washing buffer) were added to each well and the plate was left for 1 h at R.T. Then, to each washed well the substrate solution (H_2O_2/TMB) was added, developed for 30 min, and A_{450} was finally measured. (B) cdELISA was performed according by the same procedure as in (A), except that the anti-COS6 Ab, COS6, and COS6-HRP conjugates were used instead of the anti-NACOS6 Ab, NACOS6, and NACOS6-HRP conjugates, respectively. (C) cdELISA was performed according by the same procedure as in (A), except that the anti-COSM Ab, COS6, and COSM-HRP conjugates were used instead of the anti-NACOS6 Ab, NACOS6, and NACOS6-HRP conjugates, respectively.

However, in the competitive indirect ELISA (ciELISA) using the anti-COSM Ab, the detection limit was approximately 1 μg/ml (ppm), and almost the same results were obtained as in the case of the anti-NACOS6 or the anti-COS6 Ab (data not shown).

Cross-Reactivity

The concentrations of the NACOSs (NACOS2-NACOS6) which inhibited 50% of the NACOS6-HRP conjugate binding to the anti-NACOS6 Ab (IC_{50}) were 4.7, 0.039, 0.0064, 0.0085, and 0.0099 μg/ml (ppm), respectively (Fig. 3). The relative reactivities of the anti-NACOS6 Ab toward NACOS2-

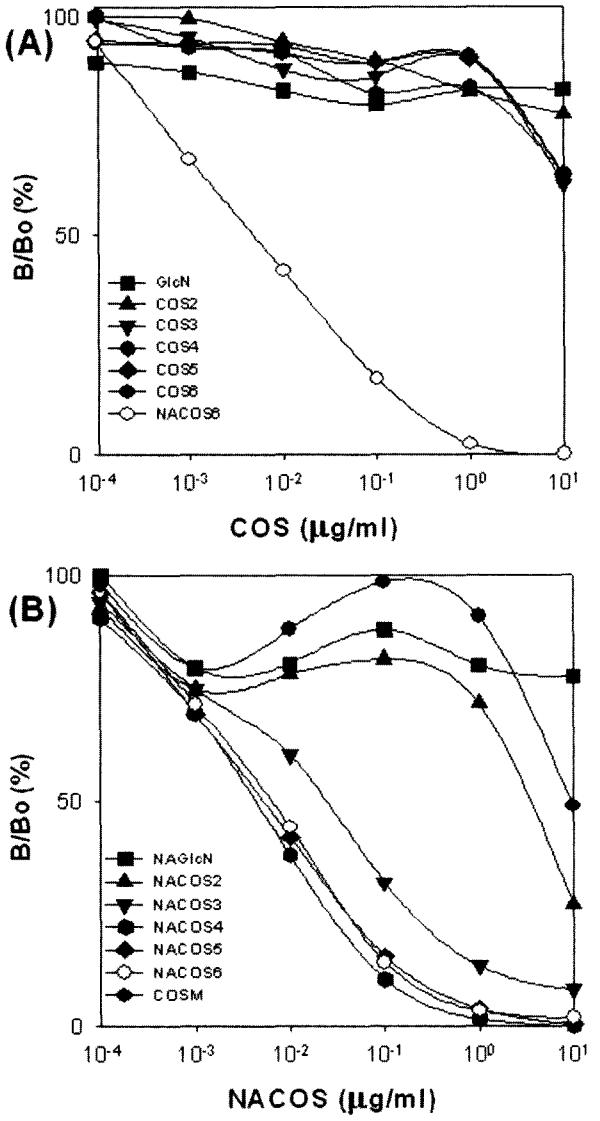

Fig. 3. Inhibition of COSs (A) and NACOSs (B) on the binding of NACOS6-HRP conjugate to the anti-NACOS6 Ab, as determined by cdELISA.
B/Bo means relative binding of NACOS6-HRP conjugate in the presence of oligosaccharides. The basic procedure was the same as in Fig. 2A.

Table 1. Cross-reactivity of the anti-NACOS6 antibody toward oligosaccharides, as determined by cdELISA.

Oligosaccharides	Oligosaccharides displacing 50% NACOS6-HRP (μg/ml)	Cross-reactivity[a] (%)
GlcN	N.D.[b]	0.00
COS2	N.D.	0.00
COS3	23	0.03
COS4	50	0.01
COS5	32	0.02
COS6	37	0.02
COSM	12.5	0.05
GlcNAc	N.D.	0.00
NACOS2	4.7	0.21
NACOS3	0.0390	25.4
NACOS4	0.0064	155
NACOS5	0.0085	116
NACOS6	0.0099	100

[a]Cross-reactivity (%)

$$= \frac{\text{Concentration of free NACOS6 displacing 50\% of NACOS6-HRP}}{\text{Concentration of oligosaccharides displacing 50\% of NACOS6-HRP}} \times 100 .$$

[b]Not determinable.

NACOS6 were 0.21, 25.4, 155, 116, and 100%, respectively, whereas those of the Ab toward GlcNAc and COSs were extremely low (Table 1), thus indicating that the anti-NACOS6 Ab recognized mainly NACOSs.

In contrast, the anti-COS6 and anti-COSM Abs were found to recognize mainly COSs. In the cdELISA using the anti-COS6 Ab, IC_{50}'s of GlcN, COS2-COS6, and COSM were 54, 0.40, 0.16, 0.08, 0.09, 0.06, and 0.10 μg/ml, respectively (Fig. 4), and the reactivities of the anti-COS6 Ab toward them were 0.1, 13.8, 34.4, 68.8, 61.1, 100, and 55.6%, respectively (Table 2).

In the cdELISA using the anti-COSM Ab, IC_{50}'s of GlcN, COS2-COS6, and COSM were 40, 0.23, 0.24, 0.20, 0.19, 0.11, and 0.11 μg/ml, respectively (Fig. 5), and the reactivities of the anti-COSM Ab toward them were 0.28, 48.9, 45.8, 55.0, 57.9, 100, and 100%, respectively (Table 3). Assuming that the reactivity of each Ab toward COS6 was 100%, the relative recognizing pattern of the anti-COSM Ab was almost same as that of the anti-COS6 Ab, except that the former recognized COS2 and COSM better than the latter did. The anti-COS6 and anti-COSM Abs produced in this study recognized mostly d.p. of COS >2. The overall cross-reactivities of the anti-COS6 and anti-COSM Abs toward NACOSs seemed to be too low to seriously affect the result of the cdELISA for COS.

Discussion

When the properties of three polyclonal antibodies (pAb) produced in this study were compared, the recognizing

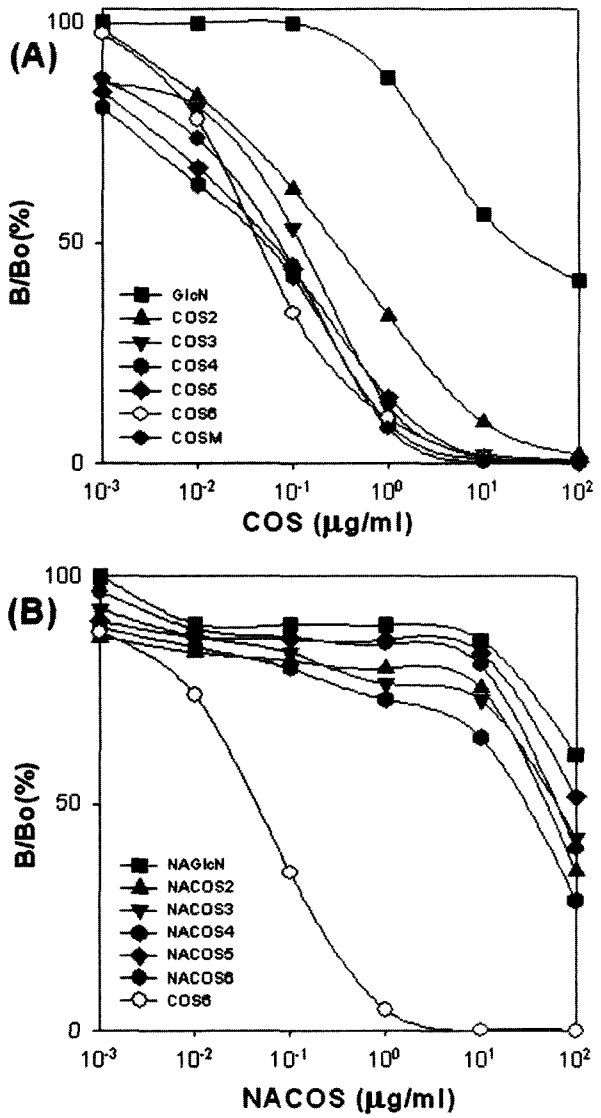

Fig. 4. Inhibition of COSs (A) and NACOSs (B) on the binding of COS6-HRP conjugate to the anti-COS6 Ab, as determined by cdELISA.
B/Bo means relative binding of COS6-HRP conjugate in the presence of oligosaccharides. The basic procedure was the same as in Fig. 2B.

pattern of the anti-COS6 Ab toward antigens was similar to that of the anti-COSM Ab. However, the pattern of the anti-NACOS6 Ab was quite different from those of the anti-COS6 and anti-COSM Abs (Tables 1-3, Figs. 3-5); The latter two Abs recognized COSs well, but not NACOSs, whereas the former Ab recognized NACOSs, but not COSs. Moreover, the anti-COS6 and anti-COSM Abs slightly recognized GlcN, a monomer of the COS6 and COSM used as a hapten for immunogen, whereas the anti-NACOS6 Ab did not recognize the monomer (GlcNAc) of NACOS6 at all [8]. The reactivities of the anti-COS6 and anti-COSM Abs toward the COSs were increased in proportion to d.p. of COSs or COS2<COS3<COS4<

Table 2. Cross-reactivity of the anti-COS6 antibody toward oligosaccharides, as determined by cdELISA.

Oligosaccharides	Oligosaccharides displacing 50% COS6-HRP (μg/ml)	Cross-reactivity[a] (%)
GlcN	54	0.10
COS2	0.40	13.8
COS3	0.16	34.4
COS4	0.08	68.8
COS5	0.09	61.1
COS6	0.06	100
COSM	0.10	55.6
GlcNAc	330	0.02
NACOS2	68	0.09
NACOS3	98	0.06
NACOS4	47	0.13
NACOS5	156	0.04
NACOS6	85	0.07

[a] $\frac{\text{Concentration of free COS6 displacing 50\% of COS6-HRP}}{\text{Concentration of oligosaccharides displacing 50\% of COS6-HRP}} \times 100$.

COS5<COS6. In contrast, the reactivities of the anti-NACOS6 Ab toward the NACOSs were decreased in proportion to the d.p. of NACOSs, or NACOS4> NACOS5>NACOS6 (>NACOS3>NACOS2).

On the other hand, the anti-COS6 and anti-COSM Abs recognized COSM to almost the same extent (IC_{50}=0.10 μg/ml and 0.11 μg/ml, respectively), but the former recognized COS3-COS6 (IC_{50}=0.06-0.16 μg/ml) better than the latter did (IC_{50}=0.11-0.23 μg/ml) (Tables 2, 3). However, when assigned that relative reactivity of each Ab was calculated on the basis of reactivity toward COS6 (100%), the cross-reactivities of both Abs toward COS4-COS5 were almost same (>50%), but those of the anti-COSM Ab toward COS2-COS3 were somewhat higher than those of the anti-COS6 Ab (Tables 2, 3). In fact, the average cross-reactivity of the anti-COS6 Ab toward COS2-COS6 and COSM was 56%, whereas that of the anti-COSM Ab was 68%, being slightly higher than the former.

These results showed that the different properties among the three Abs were due to the different immunogens which were prepared by using the different haptens and/or methods for conjugation. The degree of acetylation of the NACOS6, COS6, and COSM used as hapten were compared. While the DDA of NACOS6 and COS6 were 0% and 100%, respectively, that of the COSM was 97%, meaning that the ratio of deacetylation to acetylation of the amino group in COSM was about 30:1. In particular, COSM was the mixture of COSs which contained different d.p. of COSs. Thus, the anti-COSM Ab recognized COSM and NACOSs apparently better than the anti-COS6 Ab, even though the reactivities of the two Abs toward NACOSs were very low. Therefore it is suggested that the cdELISA using anti-COSM Ab would be more useful than the cdELISA using anti-COS6 Ab, if COS6 is used as a standard to determine COSM.

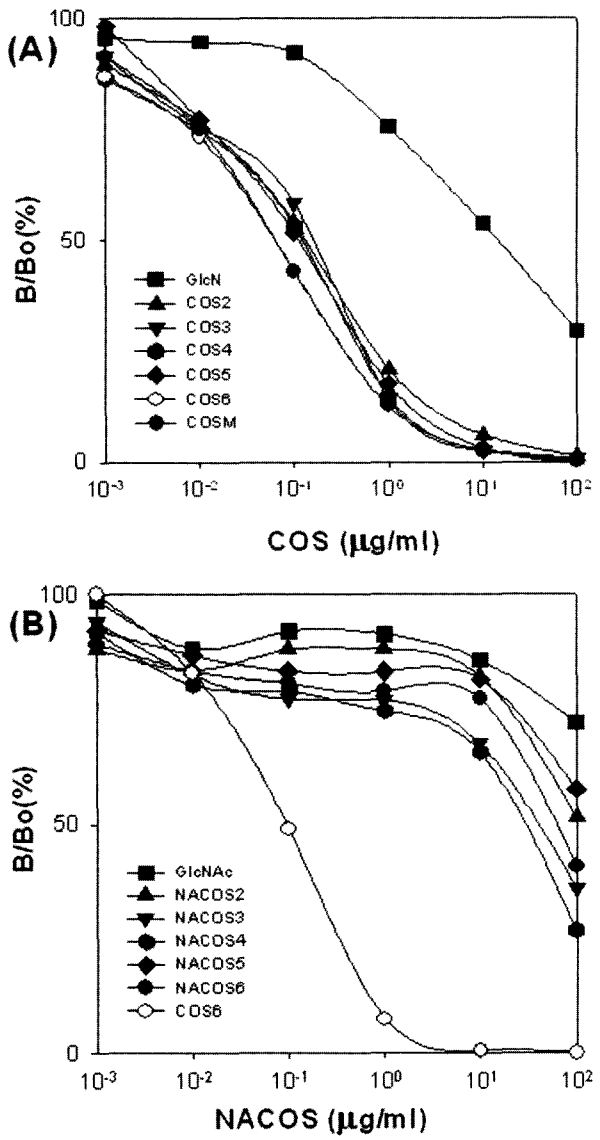

Fig. 5. Inhibition of COSs (A) and NACOSs (B) on the binding of COSM-HRP conjugate to the anti-COSM Ab, as determined by cdELISA.
B/Bo means relative binding of COSM-HRP conjugate in the presence of oligosaccharides. The basic procedure was the same as in Fig. 2C.

In summary, cdELISAs were developed, and their detection limits were compared. The detection limits of the cdELISA toward COS6 using the anti-COS6 and/or the anti-COSM Abs were about 2 ng/ml (ppb), respectively, whereas that of the cdELISA toward NACOS6 using the anti-NACOS6 Ab was about 0.2 ng/ml (ppb). Generally, the ciELISA is less sensitive than the cdELISA. The detection limit of the ciELISA toward COS6 using the anti-COSM Ab was 1 μg/ml (ppm), being about 500 times less sensitive than that of cdELISA.

Table 3. Cross-reactivity of the anti-COSM antibody toward oligosaccharides, as determined by cdELISA.

Oligosaccharides	Oligosaccharides displacing 50% COS6-HRP (μg/ml)	Cross-reactivity[a] (%)
GlcN	40	0.28
COS2	0.23	48.9
COS3	0.24	45.8
COS4	0.20	55.0
COS5	0.19	57.9
COS6	0.11	100
COSM	0.11	100
GlcNAc	3100[b]	0.004[b]
NACOS2	155	0.07
NACOS3	51	0.22
NACOS4	39	0.28
NACOS5	215	0.05
NACOS6	76	0.14

[a] $\frac{\text{Concentration of free COS6 displacing 50\% of COSM-HRP}}{\text{Concentration of oligosaccharides displacing 50\% of COSM-HRP}} \times 100$.

[b] The value was extrapolated.

Based on these results, the cdELISAs using the pAbs produced in this study could be applied to detect COSs or NACOSs in complex matrices such as foods. Furthermore, the cdELISAs using the anti-COSM and anti-NACOS6 Abs would preferably be applicable for COSs and NACOSs, respectively.

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