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Analysis of specific interactions of synthetic glycopolypeptides carrying *N*-acetyllactosamine and related compounds with lectins

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Abstract

Analysis of interactions of synthetic glycopolypeptides with lectins was performed with a biosensor based on surface plasmon resonance (SPR). A series of synthetic oligosaccharide-substituted poly(L-glutamic acid)s were immobilized on sensor surfaces via the γ-carboxyl groups of their peptide moieties by the surface thiol coupling method. Artificial glycopolypeptides: an *N*-acetyllactosamine-substituted polymer (**1**), an *N*-acetylisolactosamine-substituted polymer (**2**), a $(GlcNAc)_3$-substituted polymer (**3**), a $(GlcNAc)_2$-substituted polymer (**4**), and a *p*-aminophenyl *N*-acetyl-β-lactosaminide-substituted polymer (**5**), were used as the ligands. On analysis by SPR, surface-bound polymers **1** and **5** reacted with *Erythrina cristagalli* agglutinin (ECA), *Lycopersicon esculentum* agglutinin (LEA), *Ricinus communis* agglutinin-120 (RCA_{120}), and wheat germ (*Triticum vulgaris*) agglutinin (WGA). Polymer **2** reacted with WGA and RCA_{120}, but did not with ECA and LEA. The results indicate that β-(1→4)-linked galactosyl residues are needed for binding to ECA and LEA. Polymer **3** reacted strongly with LEA and WGA, but polymer **4** reacted strongly only with WGA. Affinity constants (K_A) for surface-bound polymer **5**–lectin interactions were also about 4–61 times as strong as those for surface-bound polymer **1**–lectin interactions. These artificial glycopolypeptides were shown to be useful as tools and probes of carbohydrate recognition and modeling in the analysis of glycoprotein–lectin interactions.

Keywords: Glycopolypeptide; Carbohydrate recognition; Surface plasmon resonance; Lectin

1. Introduction

Interactions between biomolecules are fundamental in life processes, and elucidation of these interactions in terms of structure–function relationships is crucial in understanding biological systems. The carbohydrate sequences of glycoproteins and glycolipids are information-rich, initiating and regulating many biological recognition processes [1,2], and many aspects of interactions between lectins and glycoproteins or glycopeptides

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have been studied. Conventional methods of affinity chromatography, electrophoresis, equilibrium dialysis, solid-phase binding assay, and titration calorimetric measurements have been used for the characterization of these interactions [3–5]. In case of synthetic glycopolymers, two-dimensional immunodiffusion in agar gel and inhibition of hemagglutination have generally been used to detect the interactions [6,7]. However, for kinetic measurements or for quantitative analysis of the interactions, it is usually necessary to label the molecules, either the lectins or carbohydrates [8,9]. Recently, a biosensor based on surface plasmon resonance (SPR) has been used to analyze interactions of glycoproteins, glycopeptides, and oligosaccharides with lectins in real-time without fluorescence or radioisotope labelling [10–13]. The method is rapid and sensitive, and yields rich and useful results, including kinetic parameters in studies of carbohydrate–protein interactions.

As a variety of oligosaccharide sequences containing *N*-acetyllactosamine (LacNAc) as the core structure of glycoproteins are of growing importance, much attention has been paid recently to glycopolymers carrying LacNAc residues [14–18]. In our previous study, we synthesized several kinds of glycopolypeptides carrying LacNAc and related compounds, and then analyzed their interactions with *Erythrina cristagalli* agglutinin (ECA), *Ricinus communis* agglutinin-120 (RCA_{120}), peanut (*Arachis hypogaea*) agglutinin (PNA), and wheat germ (*Triticum vulgaris*) agglutinin (WGA) by double-diffusion reaction in agar gel and in terms of inhibition of hemagglutination [18]. In the present paper, we report on the interaction analysis of immobilized synthetic glycopolypeptides with lectins by SPR, as a model for the analysis of glycoprotein–lectin interactions.

2. Experimental

Materials.—A biosensor, BIAcore 2000 (Pharmacia Biosensor AB, Uppsala, Sweden), was used to determine the biomolecular interaction between artificial glycopolypeptide and lectin.

Synthetic glycopolypeptides, poly(*N*-acetyllactosaminyl-L-glutamine-co-glutamic acid) [poly (LacNAc/Gln-co-Glu), **1**] with a degree of substitution (DS) of 44%, poly(*N*-acetylisolactosaminyl-L-glutamine-co-glutamic acid) [poly(isoLacNAc/Gln-co-Glu), **2**, DS 31%], poly(*N,N′,N″*-triacetylchitotriosyl-L-glutamine-co-glutamic acid) [poly($(GlcNAc)_3$/Gln-co-Glu), **3**, DS 38%], poly (*N,N′*-diacetylchitobiosyl-L-glutamine-co-glutamic acid) [poly($(GlcNAc)_2$/Gln-co-Glu), **4**, DS 44%], and *p*-aminophenyl *N*-acetyl-*β*-lactosaminide were prepared by our methods [18–20]. RCA_{120} and WGA were purchased from Seikagaku Corp. (Tokyo, Japan). BIAcore surfactant P20, *N*-ethyl-*N′*-(3-diethylaminopropyl)carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide, 2-(2-pyridinyldithio)ethaneamine (PDEA), NAP-10 column and sensor chip CM5 (research grade) were purchased from Pharmacia Biosensor AB. Poly(L-glutamic acid) sodium salt (molecular weight 13,600, degree of polymerization 90), 1,4-dithioerythritol, cystamine dihydrochloride, *Lycopersicon esculentum* agglutinin (LEA), and ECA were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Benzotriazol-1–yloxy-tris(dimethylamino)phosphonium hexafluorophosphate and hydroxybenzotriazole were obtained from Aldrich (Milwaukee, WI, USA). HBS buffer was prepared from 10 mM 2-hydroxyethyl piperazine *N′*-2-ethanesulfonic acid (pH 7.4), 150 mM NaCl and 0.05% BIAcore surfactant P20 in distilled water.

Analytical methods.—HPLC analysis for synthetic glycopolymers was performed with a Hitachi 6000 liquid chromatograph with a column of TSK gel $3000PW_{XL}$ (ϕ 7.8×300 mm, Tosoh Corp., Tokyo, Japan) developed with 0.2 M NaCl at a flow rate of 0.5 mL/min with a UV detector at 210 nm. ^1H and ^{13}C NMR spectra were recorded on Jeol JNM-EX 270 spectrometers in D_2O using sodium 4,4-dimethyl-4-silapentanoate as the external standard.

*Preparation of poly(*p-*aminophenyl* N-*acetyl*-β-*lactosaminide*-L-*glutamine-co-glutamic acid) [poly (LacNAc* β-p*AP/Gln-co-Glu), **5**]*.—Poly(L-glutamic acid)s (10 mg, 0.09 mmol) were dissolved in 0.2 mL of Me_2SO. A solution (0.2 mL) in Me_2SO containing benzotriazol-1-yloxy-tris(dimethylamino) phosphonium hexafluorophosphate (80 mg, 0.385 mmol) and hydroxybenzotriazole (9 mg, 0.066 mmol) was added, and the resulting mixture was stirred for 15 min at room temperature. *p*-Aminophenyl *N*-acetyl-*β*-lactosaminide (25 mg, 0.053 mmol) in Me_2SO (0.4 mL) was added and stirring was continued for 7 h under the same conditions. The solution was put immediately on a column of Sephadex G-25 (ϕ 1.0×25 cm), and eluted with 0.02 M sodium phosphate buffer (pH 7.4) containing 0.1 M NaCl at a flow rate of 1.0 mL/min.

The carbohydrate component of the elution was monitored by the phenol–H_2SO_4 method at 485 nm. Tubes 22–34 (1.0 mL/tube) were collected, concentrated using an Amicon Diaflo ultrafiltration unit equipped with a YM-3 membrane operating at 2 kg/cm^2, and lyophilized, to give **5** (15 mg).

^{1}H NMR (D_2O, 30 °C): δ 7.36 (d, 2 H, *m*-Ph), 7.08 (d, 2 H, *o*-Ph), 5.15 (d, H-1), 4.56 (d, *J* 7.3 Hz, H-1′), 4.37 (peptide α-methine NHC*H*CO), 3.60–4.09 (other protons from LacNAc unit), 2.33 (peptide γ-methylene $CHCH_2CH_2$), 2.10 (peptide β-methylene $CHCH_2CH_2$), and 2.08 (s, 3 H, NH $COCH_3$). ^{13}C NMR (D_2O, 30 °C) δ 183.99, 177.72, 176.35 and 175.88 (C=O), 157.00 (Ph carbon attached to the phenolic oxygen), 135.05 (*p*-Ph), 127.70 (*m*-Ph), 120.23 (*o*-Ph), 105.84 (C-1′), 102.80 (C-1), 81.01 (C-4), 78.27 (C-5′), 77.88 (C-5), 75.49 (C-3′), 75.20 (C-3), 73.94 (C-2′), 71.57 (C-4′), 63.97 (C-6′), 62.86 (C-6), 57.99 (C-2), 56.41 (peptide α-methine NH*C*HCO), 36.24 and 35.02 (peptide γ-methylene $CHCH_2CH_2$), 30.87 (peptide β-methylene $CHCH_2CH_2$), and 25.16 ($NHCOCH_3$).

The degree of substitution was estimated from the ^{1}H NMR area ratios at 5.15 ppm for the *N*-glycosylated H-1 proton, at 2.33 and 2.10 ppm for the peptide β- and γ-methylene protons, and 2.08 ppm for the acetyl protons, giving 36% (in molar ratio).

Immobilization of modified artificial glycopolypeptide to the sensor surface.—Before immobilization of synthetic glycopolypeptides to sensor surfaces, the polymers were treated with PDEA to convert them into thiol derivatives [21–23]. One milligram of glycopolypeptide and 5 mg of PDEA were dissolved in 1 mL of 0.1 M 2-(*N*-morpholino)ethanesulfonic acid buffer (pH 5.0) and cooled on ice. Twenty μL of 0.4 M EDC in water was added, and allowed to react on ice for 1 h. The low-molecular-weight reagents were then removed from the mixture through a NAP-10 column. The degree of modification (the molar ratio of carboxyl groups modified in the glycopolypeptide) was determined by a spectrophotometric method [21]. Then, modified glycopolypeptides were immobilized on the surfaces of CM5 sensor chips using the surface thiol coupling method. First, the sensor surface was activated by a 10 μL injection of a mixture of 100 mM *N*-hydroxysuccinimide and 400 mM EDC. Subsequently, 15 μL of 40 mM cystamine dihydrochloride in 0.1 M borate buffer (pH 8.5) and 15 μL of 100 mM 1,4-dithioerythritol in 0.1 M borate buffer (pH 8.5) were injected. Next 10–40 μL of modified artificial glycopolypeptide (200 μzg/mL in 10 mM Na-citrated buffer, pH 3.0) was injected over the surface of the sensor chip. Unreacted moieties remaining on the surface were deactivated by injection of 20 μL of 20 mM PDEA containing 1 M NaCl in 0.1 M formate buffer (pH 4.3). The immobilized amount of glycopolypeptide in RU corresponded to the difference between the initial baseline before immobilization and the final response after immobilization.

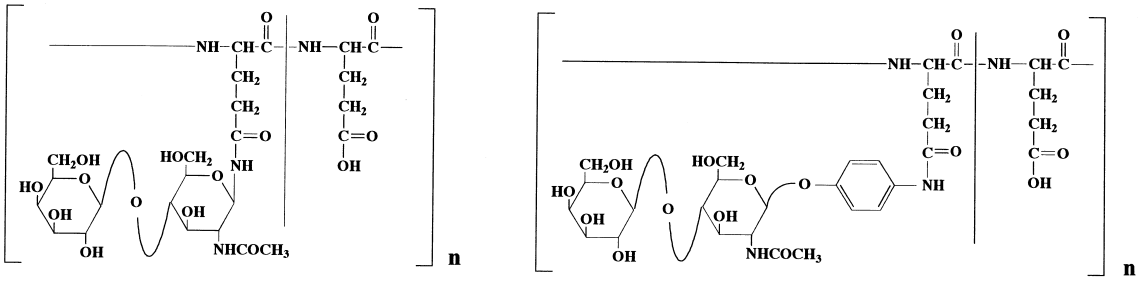
Analysis of the interaction between lectin and artificial glycopolypeptide.—Each lectin solution was injected over immobilized artificial glycopolypeptide surfaces at a flow rate of 20 μL/min. Three min after injection, HBS buffer was introduced onto the sensor surface in place of the lectin solution to start the dissociation. All sensorgrams were recorded as the changes in the SPR response at 25 °C. For measurement of the association rate constants (k_a), the data were analyzed by using several concentrations of lectin appropriately diluted with HBS buffer. Inhibition of hemagglutination caused by synthetic glycopolypeptides and their corresponding sugars were also determined according to the reported methods [18,24].

Regeneration of the biosensor surface.—The same glycopolypeptide-treated surface was used repeatedly to analyze the interactions with several different lectins. Regeneration after each binding experiment was accomplished by injection of 10 μL of 100 mM HCl, followed by extensive washing with HBS buffer.

BIAcore data analysis.—Transformation of data and kinetic parameters of interaction were made with the standard BIAevalution software (Pharmacia Biosensor AB).

3. Results

Immobilizaton of synthetic glycopolypeptides on the sensor surface.—The surface thiol coupling method [22] was used for immobilizing synthetic glycopolypeptides onto sensor chip surfaces because there are nonsubstituted carboxyl groups in these glycopolymers (Fig. 1). Glycopolymers were first treated with PDEA to introduce reactive disulfide groups. The degree of modification of polymer **1** was determined spectrophotometrically to be 2.9, which was suitable for the subsequent immobilization. Next, the modified glycopolymers



Poly(LacNAc/Gln-co-Glu) (**1**) Poly(LacNAcβ-*p*AP/Gln-co-Glu) (**5**)

Fig. 1. The structures of synthetic glycopolypeptides carrying *N*-acetyllactosamine or *p*-aminophenyl *N*-acetyl-β-lactosaminide residues.

were immobilized on the surface of a research-grade CM5 sensor chip. A typical sensorgram is shown in Fig. 2. Gradient surfaces bearing different amounts of immobilized glycopolymer were achieved by varying the injection volume of the modified polymer solution. To evaluate whether or not the immobilized glycopolypeptides interact with lectins, we monitored their interactions with appropriate lectins. The following results indicated that the immobilization procedure for the artificial glycopolypeptides was indeed suitable for the analysis.

Interactions between artificial glycopolypeptide and lectin.—Interactions with lectins were analyzed with the surface-bound glycopolypeptides using BIAcore 2000. Four lectin solutions were

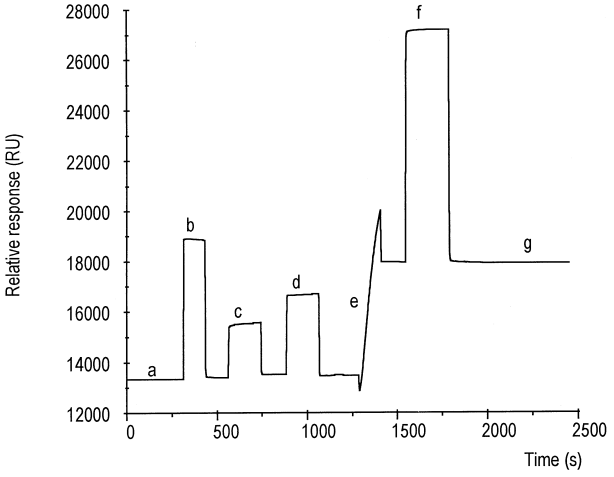


Fig. 2. One representative sensorgram of immobilization of sensor surface by modified artificial glycopolypeptide **1**. [(a) baseline signal for the unmodified sensor chip CM5; (b) injection of *N*-hydroxysuccinimide–EDC to activate the sensor surface; (c) injection of cystamine to derivatize the sensor surface; (d) injection of 1,4-dithioerythritol to reduce cystamine disulfides to thiols; (e) injection of 10 μl PDEA-modified **1** in 10 mM citrate buffer; (f) injection of PDEA–NaCl solution to deactivate excess thiol groups and remove non-covalently bound **1**; (g) the immobilized ligand corresponded to about 4400 RU.]

injected over the surface-bound polymers. The following lectins were used (their binding saccharides are given in brackets) ECA [β-Gal-(1→4)-GlcNAc, GalNAc, and Gal], LEA [$(GlcNAc)_n$ ($n \geqq 3$) and poly-*N*-acetyllactosamine], RCA_{120} (β-Gal), and WGA [$(GlcNAc)_n$] [25–27]. As shown in Fig. 3, an increase in RU from the initial baseline represents binding of the injected lectin with surface-bound glycopolymer. Polymers **1** and **5**, carrying side chains of LacNAc and *p*-aminophenyl *N*-acetyl-β-lactosaminide units, respectively, reacted with all of the lectins. Polymer **2**, carrying *N*-acetyliso-lactosamine, bound with WGA and RCA_{120}. Polymer **3**, carrying $(GlcNAc)_3$, reacted strongly with WGA and LEA, while polymer **4**, carrying $(GlcNAc)_2$, reacted with WGA but only weakly with LEA. To demonstrate whether the interactions with lectins are sugar specific or not, we injected the lectin solutions over (1) a blank surface subjected to immobilization chemistry in the absence of the artificial polymers and (2) the surface with immobilized poly(L-glutamic acid)s [28]. In the two cases, the lectins used did not show any binding. Thus, the binding specificity of each synthetic glycopolymer with the lectin is clearly indicated by a significant increase in the SPR response with the BIAcore system.

When 208 nM RCA_{120} solution was injected over a gradient surface with 710 RU of the polymer **1** immobilized in flow-cell 1, 330 RU in flow-cell 2, and 220 RU in flow-cell 3, the SPR responses for three flow-cells varied with the amounts of immobilization (Fig. 4a). Similarly, interactions between LEA and surface-bound polymer **1** were clearly detected for flow-cells 1 and 2 (Fig. 4b), but not for flow-cell 3. In this case, there was no response to either lectin, although flow-cell 3 had only a 1/3 lower polymer concentration. BIAcore technology has two shortcomings (*i*) the sensitivity does not

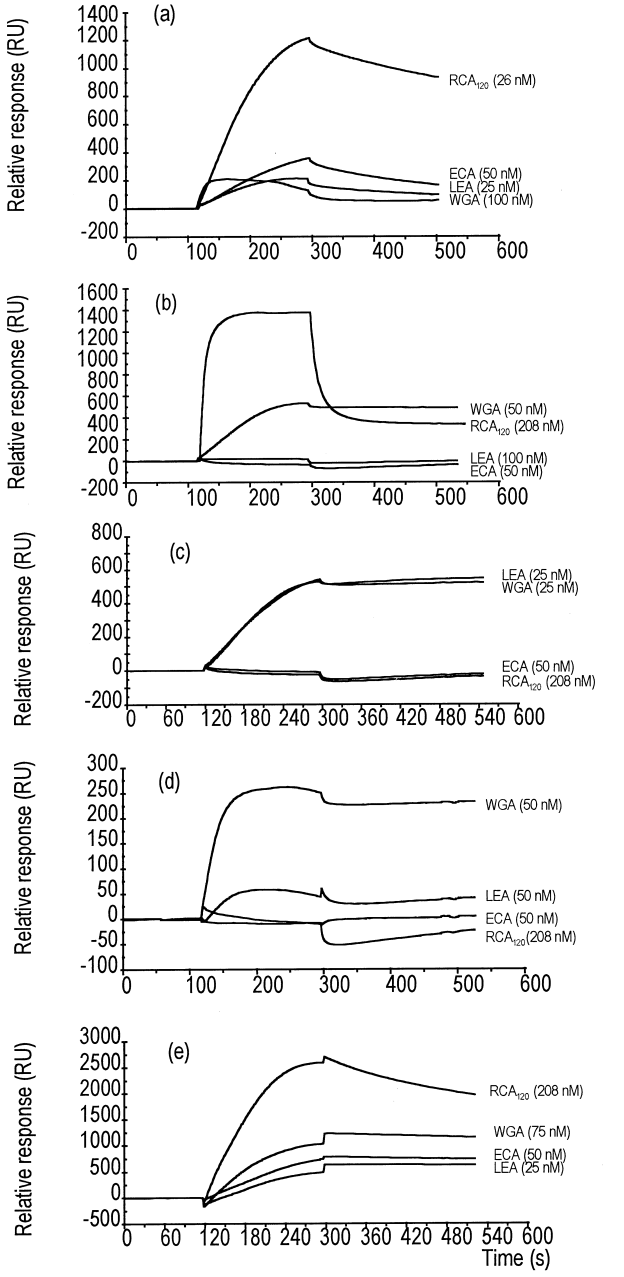


Fig. 3. Sensorgrams show interactions of synthetic glycopolypeptides with lectins. [The sensorgrams of the interactions show each association and dissociation phase as a relative response of SPR against time. (a) Glycopolypeptide **1**; (b) glycopolypeptide **2**; (c) glycopolypeptide 3; (d) glycopolypeptide **4**; (e) glycopolypeptide **5**.]

allow direct detection of low-molecular-weight analytes (< 2000 Da) and (*ii*) Iow-affinity interactions are difficult to characterize. Thus, R. Karlsson et al. recommended the use of a gradient ligand-surface to overcome such shortcomings [29]. The manner in which the amount of immobilization affects the kinetic parameters of interactions was also examined using a gradient surface. For the interaction between polymer **1** and RCA_{120}, the kinetic parameters obtained were no different when surfaces with immobilized amounts of 330, 710, and 930 RU, respectively, were used. Even at the lowest amount of immobilized glycopolymer, it was shown to be available.

Kinetic measurements of interactions of glycopolypepides with lectins.—Fig. 5 shows the sensorgrams of interactions of surface-bound polymer **1** with ECA, LEA, RCA_{120} and WGA using serial concentrations of lectin. For a subsequent injection using the same surface, the surface was regenerated at the end of each cycle with 100 mM HCl. Slopes (*ks*) of dR/d*t* versus RU, plotted against concentrations of injected lectin, were linear, as shown in Fig. 6a, and the k_a value was obtained from the slope of such a plot. Nonlinear regression analysis of appropriate parts of sensorgrams also gave good curve-fitting to the monoexponetial association model (Fig. 6b), and k_a values determined by both methods were in excellent agreement. Table 1 shows the kinetic parameters of some interactions of the artificial glycopolypeptides with lectins. In the case of WGA, the different affinities for polymers **2–4** could be attributed to the large differences of k_d values, whereas the differences in k_a values were relatively small. Polymer **5** had relatively higher affinities with the four lectins than did polymer **1**, because of the difference in the value of k_d. This shows that the affinity of surface-bound glycopolymers with lectins is primarily dominated by the dissociation rate.

These quantitative analyses were compared with the analytical results obtained by hemagglutination inhibition (Table 2) as supplemental data. The results show that the interactions of glycopolypeptides with lectins as detected by hemagglutination inhibition were all detected by BIAcore in real-time. In addition, interactions of polymers **1** with LEA and WGA, which were not observed by inhibition of hemagglutination, were detected with BIAcore. It is suggested that the method is quite sensitive for analysis of interactions between glycopolymers and lectins.

4. Discussion

With SPR detection, it was possible to immobilize synthetic glycopolymers and analyze the interactions of the surface-bound glycopolymers with

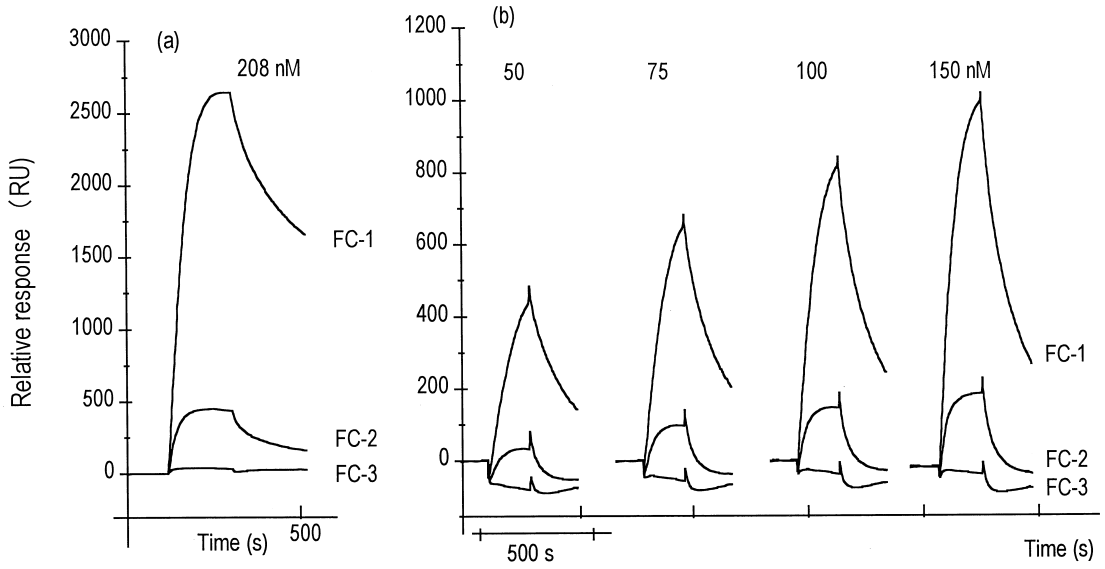


Fig. 4. The sensorgrams show interactions of a gradient surface of immobilized glycopolypeptide **1** with (a) RCA_{120} and (b) LEA.

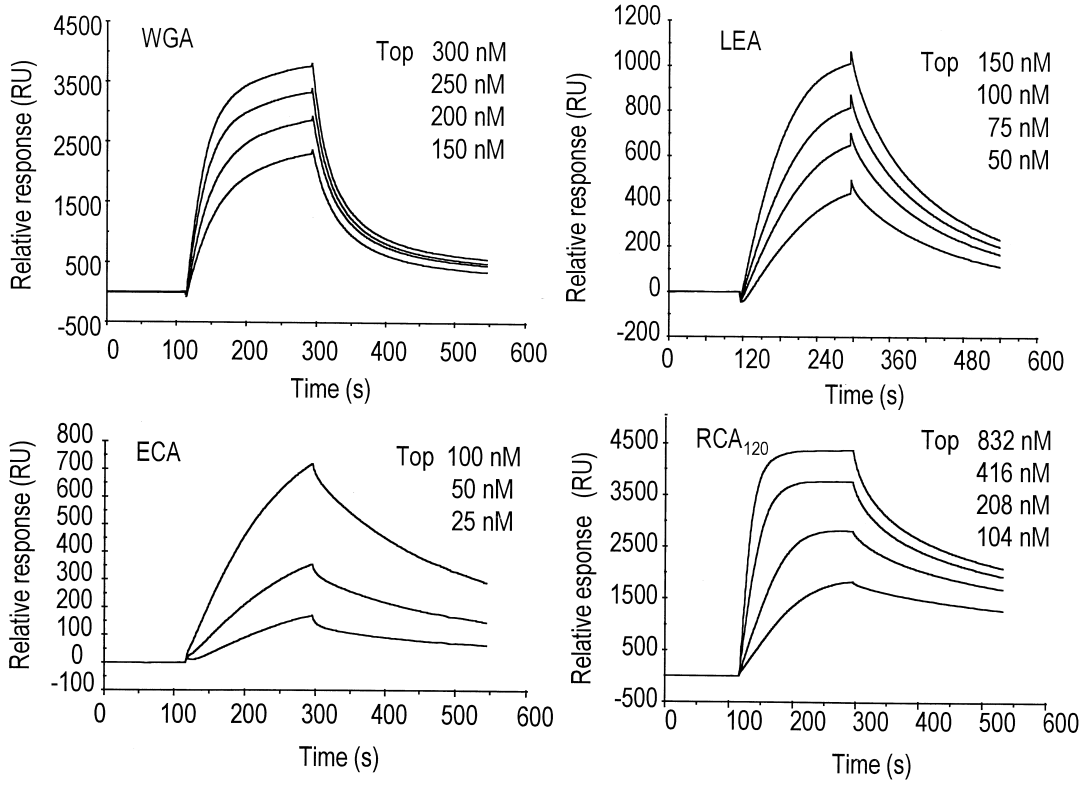


Fig. 5. Sensorgrams show interactions of synthetic glycopolypeptide **1** with ECA, LEA, RCA_{120}, and WGA lectins.

injected lectins. This is the first report using synthetic glycopolymers as biosensor ligands. The surface-bound polymer **1** reacted with four lectins, whereas interactions of polymer **1** with LEA and WGA were not detected by hemagglutination inhibition. Some researchers have reported that the chain length of poly-*N*-acetyllactosamine-type oligosaccharides of natural glycoproteins influences the binding with LEA [30,31]. Thus, the long poly-*N*-acetyllactosamine chains show high affinity with LEA, whereas the short one show low affinity. Our results show that BIAcore enabled detection of the binding of LEA and WGA with the surface-bound polymer **1** carrying side chains

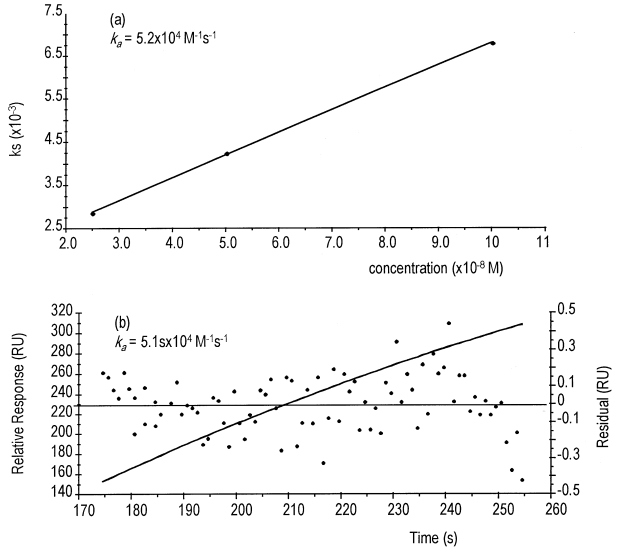


Fig. 6. Kinetic parameters of interactions between glycopolypeptide **1** and ECA. [(a) Linear regression analysis of *ks* versus C plot for k_a determination, and (b) nonlinear analysis of association phase by fitting to BIA evalution A + B = AB model.]

of the non-repeating LacNAc unit alone in the glycopolymer. Some reports indicate that the presence of hydrophobic groups in the sugar side-chain in artificial glycopolymers enhances the interaction with WGA [6,17,32–34]. Therefore, instead of polymer **1**, glycopolymer **5** carrying *p*-aminophenyl *N*-acetyl-β-lactosaminide units having the hydrophobic aglycon in the side chain, was synthesized and used for interaction analysis with lectin. The results showed that surface-bound polymer **5** reacted with all of the lectins tested. The K_A values of polymer **5** were 3–60 times higher than those of polymer **1**. This result is consistent with the result that polymer **5** inhibited the four lectins more strongly on hemagglutination than did polymer **1**. Stronger interactions of polymer **5** with LEA and WGA as compared to polymer **1** were also verified by this analytical method.

Table 1
Kinetic parameters obtained from the interactions of artificial glycopolypeptides with lectins by use of a biosensor

Glycopolypeptide	Lectin	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_A (M^{-1})
Polymer **1**	WGA	6.3×10^4	8.0×10^{-3}	7.9×10^6
	LEA	5.4×10^4	6.4×10^{-3}	8.5×10^6
	ECA	5.2×10^4	5.1×10^{-3}	1.0×10^7
	RCA_{120}	3.1×10^4	3.8×10^{-3}	8.2×10^6
Polymer **2**	WGA	6.0×10^5	2.3×10^{-3}	2.6×10^8
	RCA_{120}	3.4×10^4	3.2×10^{-3}	1.1×10^7
Polymer **3**	WGA	5.8×10^5	3.7×10^{-5}	1.6×10^{10}
	LEA	5.7×10^5	2.9×10^{-5}	2.0×10^{10}
Polymer **4**	WGA	6.9×10^5	6.5×10^{-4}	1.1×10^9
	LEA	— [a]	—	—
Polymer **5**	WGA	5.2×10^4	9.7×10^{-4}	5.3×10^7
	LEA	5.8×10^4	1.1×10^{-4}	5.2×10^8
	ECA	6.9×10^4	1.6×10^{-3}	4.4×10^7
	RCA_{120}	7.0×10^4	1.7×10^{-3}	4.1×10^7

[a] Kinetic parameters were not calculated because of the weak interaction of the polymer with the lectin.

The kinetic parameters of the interactions of surface-bound polymer **1** with WGA were compared with those of surface-bound polymer **2**. The k_a value of surface-bound polymer **2** was about 8.5 times higher than that of surface-bound polymer **1**, and the k_d value was about 2.5 times lower. As a result, the K_A value of surface-bound polymer **2** was about 32 times higher than that of surface-bound polymer **1**. In hemagglutination inhibition, polymer **2**, carrying *N*-acetylisolactosamine (an artificial oligosaccharide), inhibited WGA more strongly than did polymer **1**. This was also the case for BIAcore analysis. Thus, the binding mode with WGA is shown to be greatly influenced by the position of the terminal galactosyl group in the side-chain of the two glycopolymers. The interactions of lectins (RCA_{120} and *Sambucus sieboldiana* agglutinin) with surface-bound fetuin and asialofetuin, natural glycoproteins which have plural LacNAc chains, have been made with BIAcore [13,35]. The k_a value of RCA_{120} for asialofetuin ($9.7\times10^4\,M^{-1}$) was similar to that obtained for polymer **5**, whereas the k_d value ($5.0\times10^{-4}\,M^{-1}\,s^{-1}$) a little lower, although the immobilization method for attaching the ligand to the surface of the sensor chip was different for each case. The present artificial glycopolymers, having well-defined sugar units, should be quite useful as models for defining the interaction between glycoproteins and lectins.

Furthermore, on analysis in SPR, these synthetic glycopolypeptides could be used to compare the binding ability of WGA and LEA lectins toward the linear $(GlcNAc)_n$ repeating-unit. Both surface-bound polymers **3** and **4** showed high affinity with WGA, but the latter showed much lower with LEA than did the former. Similar results were obtained by tests of hemagglutination inhibition where both polymers **3** and **4** inhibited WGA strongly, while the latter did not inhibit LEA. In our previous report, a glycopolypeptide carrying a single GlcNAc unit as the side chain also showed strong inhibitory activity to WGA [18]. The binding properties of lectins accorded with the specificities

Table 2
Inhibition of lectin [a]-induced hemagglutination by glycopolypeptides and sugars

Inhibitor	Minimum inhibitory concentration [mg/mL (M [b])]			
	WGA	LEA	ECA	RCA_{120}
1 (44%) [c]	N.I. [d]	N.I.	0.13 (1.9×10^{-4})	2.50 (3.8×10^{-3})
LacNAc	N.I.	N.I.	0.15 (3.9×10^{-4})	0.30 (1.6×10^{-3})
2 (31%)	0.078 (9.9×10^{-5})	N.I.	N.I.	2.50 (3.2×10^{-3})
IsoLacNAc	N.I.	N.I.	N.I.	1.20 (3.1×10^{-3})
3 (38%)	2.0×10^{-4} (2.1×10^{-7})	7.6×10^{-5} (8.0×10^{-8})	N.I.	N.I.
$(GlcNAc)_3$	0.16 (2.6×10^{-4})	0.49 (7.7×10^{-4})	N.I.	N.I.
4 (44%)	3.9×10^{-4} (5.6×10^{-7})	N.I.	N.I.	N.I.
$(GlcNAc)_2$	1.25 (2.9×10^{-3})	N.I.	N.I.	N.I.
5 (36%)	1.25 (1.5×10^{-3})	0.313 (3.8×10^{-4})	0.063 (7.5×10^{-4})	0.625 (7.5×10^{-4})
β LacNAc-*p*NP	N.I.	N.I.	0.055 (1.1×10^{-4})	0.220 (4.3×10^{-4})

[a] The concentration of lectin used was fourfold the minimum to cause hemagglutination.
[b] Concentration of sugar unit.
[c] Degree of substitution of sugar in glycopolypeptide as percentage.
[d] N.I. means that no inhibition occurred by 10 mg/mL of sugar or 5 mg/mL of glycopolypeptide. But in case of β LacNAc-*p*NP, N.I. means that hemagglutination was not inhibited by 1.25 mg/mL.

of the two lectins, where WGA recognizes GlcNAc units, whereas LEA shows stronger binding towards chito-oligosaccharide $(GlcNAc)_n$ ($n\geqq3$) units [25,27].

In conclusion, the immobilization of artificial glycopolypeptides onto the surface of a sensor chip is shown to be an effective procedure for analyzing specific interactions of surface-bound glycopolypeptides with lectins. The glycopolypeptides are useful not only as probes for carbohydrate recognition, but also as models in glycoprotein–lectin interactions.

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