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# Synthesis of Polyrotaxane-biotin Conjugates and Surface Plasmon Resonance Analysis of Streptavidin Recognition

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**Abstract** A polyrotaxane-biotin conjugate was synthesized and its interaction with streptavidin measured using surface plasmon resonance (SPR) detection. A biodegradable polyrotaxane in which *ca.* 22 molecules of α-cyclodextrins (α-CDs) were threaded onto a poly(ethylene oxide) chain ($M_n$: 4,000) capped with benzyloxycarbonyl-L-phenylalanine was conjugated with a biotin hydorazide and 2-aminoethanol after activating the hydroxyl groups of α-CDs in the polyrotaxane using *N,N'*-carbonyldiimidazole. The results of the high-resolution $^1$H-nuclear magnetic resonance ($^1$H-NMR) spectra and gel permeation chromatography of the conjugate showed that *ca.* 11 biotin molecules were actually introduced to the polyrotaxane scaffold. An SPR analysis showed that the binding curves of the biotin molecules in the conjugate on the streptavidin-deposited surface changed in a concentration dependent manner, indicating that the biotin in the conjugate was actually recognized by streptavidin. The association equilibrium constant ($K_a$) of the interaction between the conjugate and streptavidin tetramer was of the order $10^7$. These results suggest that polyrotaxane is useful for scaffolds as a polymeric ligand in biomedical fields.

*Keywords*: polyrotaxanes, biotin, conjugate, multivalent ligands, surface plasmon resonance

## INTRODUCTION

Receptor-ligand interactions in living systems play a major role in cell-cell, cell-protein and cell-saccharide interactions [1]. Most previous investigations on receptor-ligand interactions have focused on the interactions of molecules with single receptor binding sites aiming at understanding the mechanism and discovering natural or non-natural ligands. However, these interactions, especially lectin binding with carbohydrate ligands, are relatively weak (association constant, $K_a \sim 10^3$-$10^4$ $M^{-1}$), and thus difficult to reconcile with the strong and specific interactions between the receptor and ligands in biological events [2,3]. Due to this inconsistency, many biological scientists have speculated that multiple interactions may cooperate in each biological recognition event to produce such strong binding properties. Multivalent ligands are termed as ligands that display multiple copies of recognition elements. The most advanced research on multivalent ligands is related to protein-carbohydrate interactions because researchers are interested to mimic the protein-carbohydrates in Mother Nature in a multivalent fashion. Non-natural carbohydrate-bearing polymers, forms of synthetic multivalent ligands, have been studied since the 1980's to identify the molecular mechanism of physiological multivalent interactions that are structurally heterogeneous [4-9].

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By using synthetic approaches for polymer scaffolds, many of the characteristics of multivalent ligands, such as the number of ligands (valency), orientation of receptor binding sites, flexibility, size, and shape, can be systematically varied [5,8-10]. Kissling *et al.* applied ring–opening metathesis polymerization (ROMP) to generate collections of multivalent saccharide displays in which the number of repeating units can be systematically varied [9,10]. Baker, Jr. *et al.* studied the effect of ligand architecture, including dendrimers, comb-branched copolymers, dendrigraft copolymers and linear-dendron architectural copolymers, on inhibitory activity and specificity [8]. From another perspevtive, antibiotic-introduced multivalent polymers have been proposed as strong inhibitors of bacterial infections [11-14] Arimoto *et al.* reported that a multivalent polymer of vancomycin, synthesized by ROMP, showed enhanced antibacterial activity against vancomycin-resistant bacteria [14]. They also suggested that multivalent polymers may be promising tools in the fight against multi-resistant bacteria. Accordingly, the design of the scaffold structure is important not only for clarifying the multivalent mechanism in nature but also for designing new drugs, such as multivalent viral and toxin inhibitors.

Supramolecular design for biological functions has become a fascinating field of biomaterials and biotechnologies [15]. Supramolecular-structured polymers, including dendrimers [16], polymeric micelles [17] and polyrotaxanes [18], have been studied as potential biomedical devices for drug delivery systems. The current au-

thors have investigated biodegradable polyrotaxanes in which a lot of chemically modified α-cyclodextrins (α-CDs) are threaded onto a poly(ethylene oxide) (PEO) chain capped with an amino acid or oligopeptide via biodegradable linkages [18-22]. The most attractive characteristics of polyrotaxanes involve i) supramolecular dissociation triggered by the hydrolysis of terminal liable linkages [19,20,22], ii) the introduction of biological ligands or functional groups into many of the hydroxyl groups of α-CDs in polyrotaxanes [21,23] and iii) the rod-like structure [24]. It would appear that these characteristics are promising when considering new types of drug delivery systems. As for the latter two characteristics, many hydroxyl groups can be useful for introducing a lot of ligands along with the rod-like structure of polyrotaxanes. As such this facilitates dual-functions in new biomaterials: controlled multivalency and biodegradation.

The objective of this study is to synthesize a polyrotaxane-biotin conjugate and evaluate its potential for new multivalent ligands using the surface plasmon resonance (SPR) technique. Biotin and streptavidin were selected as the ligand and receptor, respectively, because i) the affinity of streptavidin to biotin ($K_a \sim 10^{14}$-$10^{15}$ $M^{-1}$) [25] is powerful enough to detect a single interaction between the ligands in the conjugates and the receptor, and ii) this combination can be applied to new biosensors, including SPR, that detect not only ligand-receptor interactions [26-28] but also interfacial conformational and structural changes in biomacromolecules [29]. The binding and dissociation kinetics of the interaction between biotin in the conjugate and streptavidin were determined from the results of an SPR analysis, and the effect of the polyrotaxane scaffold on the biotin recognition by streptavidin is discussed.

## MATERIALS AND METHODS

### Materials

The α-cyclodextrin (α-CD) was purchased from Bio-Research Corporation of Yokohama, Yokohama, Japan. The α-(3-Aminopropyl)-ϖ-(3-aminopropyl) polyoxyethylene (PEO-BA : $Mn$ = 4000) was kindly supplied by Sanyo Chemical Co. Kyoto, Japan. The benzyloxycarbonyl-L-phenylalanine (Z-L-Phe), 2-aminorthanol, *N,N'*-carbonyldiimidazole, formic acid and *d*-biotin were purchased from Wako Pure Chemical Co. Ltd., Osaka, Japan. The *N*-hydroxysuccinimide and 1-hydroxybenzotriazole (HOBt) were purchased from Peptide Institute, Inc., Osaka, Japan. The phosphate buffered saline (pH 7.4) containing 0.05 v/v % of Tween 20 (PBS/T) (10 mM sodium phosphate, 2.7 mM potassium chloride, 138 mM sodium chloride and 0.05% Tween 20) was prepared by the dissolution of PBS/T powder purchased from Sigma Chemical Co., St. Louis, USA and kept at –4°C until use. The EZ-Link™ biotin hydrazide and ImmunoPure® streptavidin were purchased from PIERCE, Rockford, USA. The biotin cuvette for the interaction analysis system (IAsys) was purchased from Affinity Sensors Cambridge, Inc., UK. The dimethylsulfoxide (DMSO) were purchased from Wako Pure Chemical Co. Ltd., Osaka, Japan, and distilled by the usual method. The DMSO for the high performance liquid chromatography (HPLC) was purchased from Kishida Chemical Co., Osaka, Japan and used as is. All other chemicals used were of reagent grade.

### Synthesis of Polyrotaxane-biotin Conjugate (Scheme 1)

A polyrotaxane in which many α-CDs were threaded onto a PEO chain capped with Z-L-Phe was prepared according to previous papers by the current authors [19,21,24]. Briefly, an inclusion complex of α-CDs and PEO-BA was prepared by simply mixing a saturated aqueous solution of α-CDs with a PEO-BA aqueous solution [30]. Then the succinimide ester of Z-L-Phe, prepared by a condensation reaction of Z-L-Phe and *N*-hydroxysccinimide, was allowed to react with the terminal amino groups in the inclusion complex in DMSO. The chemical structure was characterized by 750 MHz $^1$H-NMR using a FT-NMR spectrometer (Varian FT-NMR Gemini750, Palo Alto, U.S.A). The number of α-CDs was determined to be *ca.* 22 from the $^1$H-NMR spectra based on comparing the integration of the signals at 4.75 ($C_1H$ of α-CD) with those at 3.49 ($CH_2CH_2O$ of PEO).

The obtained polyrotaxane (13.6 μmol, hydroxyl groups: 6.1 mmol) was dissolved in dry DMSO (20 mL), then CDI (30.7 mmol) was added to the solution, which wan then stirred for 3 h at room temperature under a nitrogen atmosphere. The reaction mixture was slowly poured into excess ether, and the precipitate filtered and dried *in vacuo* at room temperature to obtain a CDI-activated polyrotaxane. The activation of the hydroxyl groups in the polyrotaxane was confirmed by the colorimetric determination of imidazole (absorbance at 207 nm) after the alkaline hydrolysis of the *N*-acyl imidazole groups.

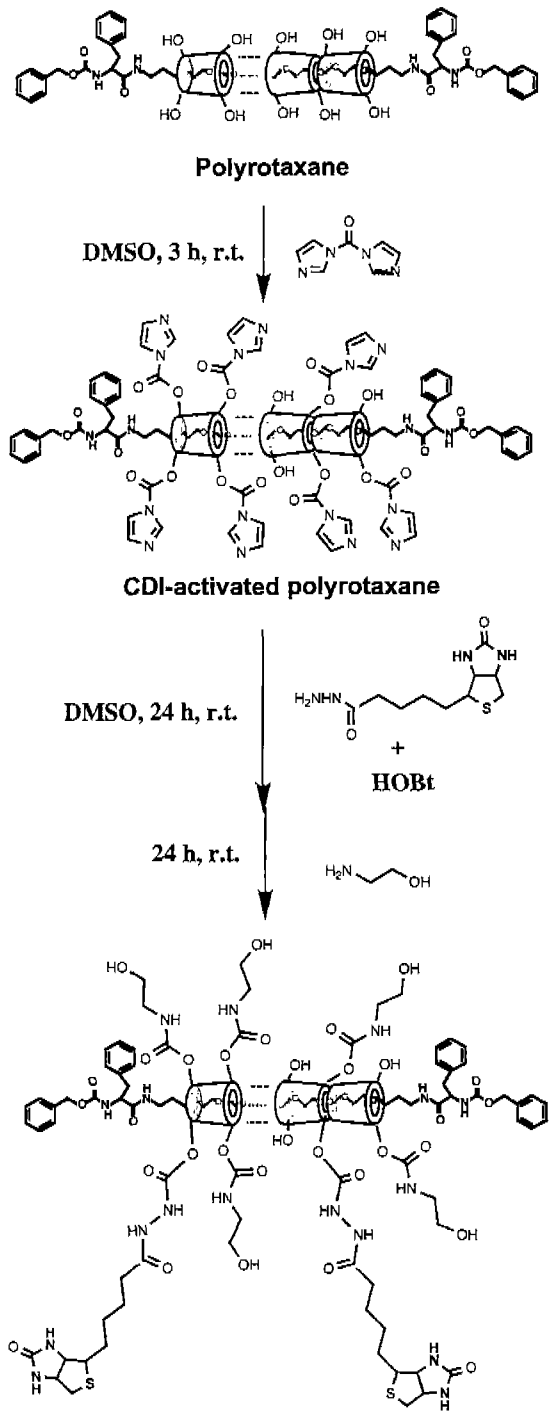
The CDI-activated polyrotaxane (number of α-CDs per polyrotaxane molecule: 22, *N*-acyl imidazole groups per polyrotaxane molecule: 0.24 mmol) was dissolved in dry DMSO (2 mL), and biotin hydrazide (0.24 mmol) and HOBt (0.24 mmol) were added to the solution under a nitrogen atmosphere. The mixture was stirred for 24 h at room temperature, then 2-aminoethanol (9.9 mmol) was dropped into the reaction mixture, followed by stirring for a further 24 h under the same conditions. After the reaction, the resulting solution was dialyzed against water (Spectra/Por® MWCO; 1,000) and lyophilized to obtain a polyrotaxane-biotin conjugate. The conjugate was characterized by a 750 MHz $^1$H-NMR and gel permeation chromatography (GPC, Colmun; TSKgel G3000$H_{HR}$ + G5000 $H_{HR}$, Tosoh, Co., Tokyo, Japan, Eluent: DMSO, Flow rate: 0.8 mL/min, and Detection: optical rotation, OR-990, Japan Spectroscopic Co., Tokyo, Japan).

Yield: 34 mg. $^1$H-NMR (DMSO-$d_6$, ppm): δ 9.39 (d,

$J$=2.3 Hz, 2H x 11, -OCONH-NHCO- of immobilized linkages), 7.38-7.16 (brm, 10H × 2, aromatics of Z-L-Phe), 7.15-6.80 (brm, 1H × 104, -OCONH- of immobilized linkages of hydroxyethyl carbamoyl groups), 6.40, 6.34 (s, 2H × 11, NH of biotin), 4.89 (brm, 6H × 20, $C_1H$ of α-CD), 4.25-3.30 (brm, 42H × 20, $O_6H$, $C_3H$, $C_5H$, $C_6H_2$, $C_4H$ and $C_2H$ of α-CD), 3.51 (s, 4H × 90, $CH_2CH_2O$ of PEO), 3.04 (brm, 4H × 104, $CH_2$ of hydroxyethyl carbamoyl groups), 2.82 (dd, $J$=4.5, 7.5 Hz, 1H × 11, $C_\varepsilon H$ of biotin), 2.58 (d, $J$=12.8 Hz, 1H × 11, $C_\varepsilon H'$ of biotin), 2.08 (m, 2H × 11, $C_\alpha H$ of biotin), 1.63-1.23 (m, 6H × 11, $C_\beta H/C_\gamma H/C_\delta H$ of biotin). The number of α-CDs and immobilized biotin was determined from the 750 MHz $^1$H-NMR spectra.

### SPR Analysis

The SPR experiments were carried out using an IAsys instrument (IAsys Auto+, Affinity Sensors Cambride Inc., UK) that quantifies a wide range of biomolecular interactions based on a resonant mirror biosensor [31-33]. The IAsys instrument temperature was set at 25°C. The resonant layer of the biotin cuvette was washed with 40 μL of PBS/T and allow to settle for 10 min for equilibration. During this equilibration, streptavidin was dissolved in PBS/T (2 mg/mL). PBS/T containing streptavidin (20 μL) was added to the PBS/T in the cuvette and allowed to settle for 10 min to facilitate binding on the biotin-immobilized surface. After washing the cuvette with 50 μL of PBS/T three times, the cuvette was left to stand for 3 min to stabilize the base line. The density of the deposited streptavidin was calculated from the resulting sensorgram based on the IAsys calibration curve (1 ng of bound streptavidin/mm$^2$ of the biotin-immobilized surface is equivalent to 600 arc sec). After equilibrating the streptavidin-deposited surface with 45 μL of PBS/T, 5 μL of the polyrotaxane-biotin conjugate dissolved in PBS/T (concentration; 50 nM biotin in the conjugate) was added to the PBS/T in the cuvette, then the binding was monitored for 10 min. Thereafter the cuvette was washed with 50 μL of PBS/T, and any dissociation was monitored for an additional 5 min. Finally, 1 M formic acid was added to the surface for 1 min to break the biotin-streptavidin bindings, then the cuvvete was washed with PBS/T three times. The same procedure was then carried out with various polyrotaxane-biotin conjugate solution conditions (1 nM, 10 nM, and 50 nM after the addition of 1 mM *d*-biotin). The resulting sensorgrams were analyzed using the Fastfit software of IAsys to determine the kinetic parameters.




**Scheme 1.**

## RESULTS AND DISCUSSION

### Polyrotaxane-biotin Conjugate

In order to introduce biotin molecules to the polyrotaxane scaffold, the hydroxyl groups of α-CDs in the polyrotaxane were activated by CDI to react with the hydrazide groups of the biotin hydrazide. After the activation reaction, the number of α-CDs in the polyrotaxane was calculated to be *ca.* 22, which was similar to the results in previous papers by the current authors [22, 23]. The degree of activation was calculated to be *ca.* 10 per one α-CD molecule, thus the total activation number per polyrotaxane molecule was *ca.* 220. This result

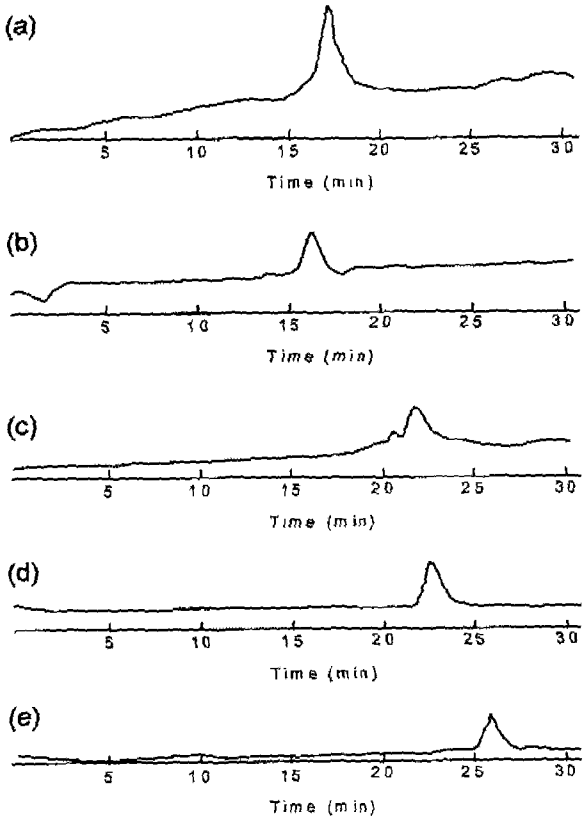



**Fig. 1.** GPC traces of (a) polyrotaxane-biotin conjugate, (b) hydroxyethylcarbamoyl-polyrotaxane, (c) α-CD-biotin conjugate, (d) α-CD and (e) *d*-biotin. Column; TSKgel G3000$H_{HR}$ + G5000 $H_{HR}$, eluent: DMSO, Flow rate: 0.8 mL/min, and detection: optical rotation.

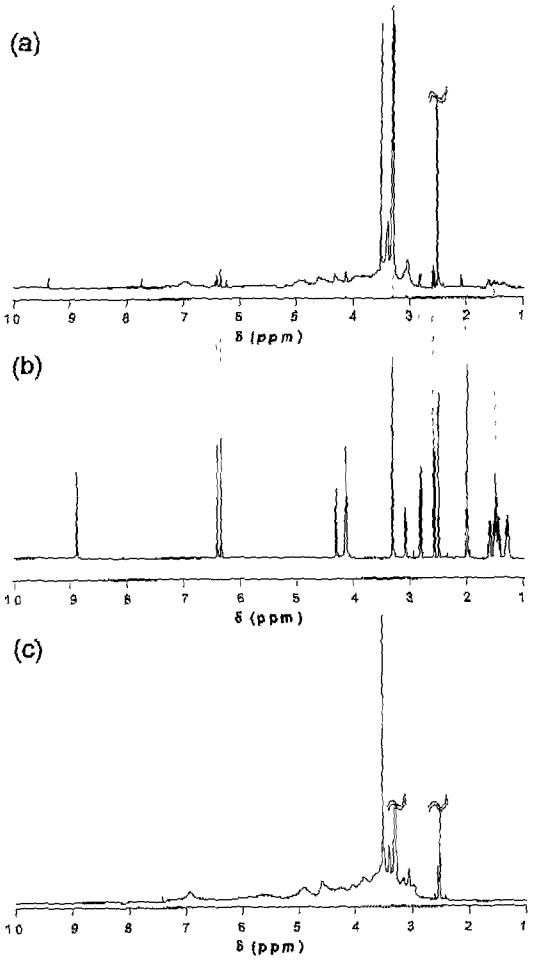



**Fig. 2.** 750 MHz $^1$H-NMR spectrum of (a) polyrotaxane-biotin conjugate, (b) *d*-biotin and (c) hydroxyethylcarbamoyl-polyrotaxane in DMSO-$d_6$.

indicates that hundreds of biotin can be theoretically introduced to the polyrotaxane scaffold. After the reaction with biotin hydrazide, the resulting product was found to be water-insoluble. It is known that hydrogen bonding between hydroxyl groups of α-CDs in polyrotaxanes causes limited water solubility, whereas the elimination of hydrogen bonding by chemical modifications, such as hydroxypropylation [19], can drastically improve the water solubility of a polyrotaxane. The reduced water-solubility after introducing biotin, a water-soluble ligand, appeared to be due to the association of alkyl chains in biotin. Based on this reason, the chemical modification of α-CDs with 2-aminoethanol (hydrxyethylcarbamoylation) was also carried out. As expected, the solubility of the polyrotaxane increased after the reaction. The polyrotaxane conjugated with biotin hydrazide and 2-aminoethanol was characterized by GPC and $^1$H-NMR spectroscopy. Fig. 1 shows the results in GPC charts of the purified conjugate, hydroxyethylcarbamoyl-polyrotaxane, α-CD-biotin conjugate (the number of biotin per α-CD: 0.6), α-CD, and *d*-biotin. The peak attributed to the conjugate was detected as single peak, elution time of which was significantly shorter than that of the α-CD-biotin conjugate, α-CD and *d*-biotin. Furthermore, the elution time of the conjugate was very similar to that of hydroxyethylcarbamoyl-polyrotaxane. These results indicate that the product obtained was a polyrotaxane derivative without any contamination in its constituents. In order to confirm the chemical compositions of the polyrotaxane derivative, the $^1$H-NMR spectrum was analyzed in comparison with those of biotin hydrazide and hydroxyethylcarbamoyl-polyrotaxane (Fig. 2). The peaks attributed to *d*-biotin and hydroxyethylcarbamoyl-polyrotaxane were confirmed. The peak attributed to the hydrazide groups (δ = 8.91 in Fig. 2(b)) was found to shift to lower magnetic fields (δ = 9.39 in Fig. 2(a)). This peak shift revealed that the *d*-biotin hydrazide was introduced to the hydroxyl groups of α-CDs in the polyrotaxane via carbamoyl linkages. As such the polyrotaxane-biotin conjugate was synthesized and the supramolecular structure maintained after biotin immobilization. The number of α-CDs, biotin, and hydroxyethylcarbamoyl groups per one polyrotaxane molecule was calculated to be *ca.* 20, 11, and 104 based on

the $^1$H-NMR spectrum, respectively, thereby indicating that one biotin molecule was introduced to every two α-CD molecules.

The conformation of the synthesized polyrotaxane-biotin conjugate under aqueous conditions was analyzed by two-dimensional nuclear Overhauser effect spectroscopy (2D NOESY). There were no correlated peaks between the peaks of *d*-biotin (NH, $C_{\alpha\text{-}\delta}H$, $C_\varepsilon H$, $C_\varepsilon H'$, $C_\zeta H$, $C_\zeta H'$) and hydroxyethylcarbamoyl-polyrotaxane (aromatics of Z-L-Phe, $O_6H$, $C_5H$, $C_6H_2$, $C_4H$, $C_3H$, $C_2H$ and $C_1H$ of α-CD, $CH_2CH_2O$ of PEO, and $CH_2$ of hydroxyethylcarbamoyl groups), although several correlated peaks between the glucose units of α-CDs were observed, presumably due to configurational changes as a result of the conjugation (data not shown). This result suggests that the biotin molecules in the conjugate were exposed to an aqueous environment.

## Effect of Biotin Conjugation with Polyrotaxane on Streptavidin Tecognition

As described in materials and method section, streptavidin tetramer was deposited on the biotin-immobilzed IAsys cuvette. Based on the resulting sensorgram, the density of the deposited streptavidin was calculated to be $2.5 \times 10^{-5}$ nmol/mm$^2$. This value of density means that streptavidin tetramer was deposited on every 64.2 nm$^2$, indicating that the average distance between two adjacent streptavidin tetramers on the surface was *ca.* 8.0 nm. The size of streptavidin tetra-mer is assumed to be 5.5 nm [25], as such the distribution image of the streptavidin-deposited surface is shown in Fig. 3. Since the depth of α-CD is 0.7 nm and the stoichiometric number of α-CDs in a PEO chain (*Mn*: 4,000) is *ca.* 45 [19], the length of the rod of the polyrotaxane was theoretically assumed to be 32 nm. Considering the threading number of α-CDs in the conjugate *(ca.* 20) and the surface density of the deposited streptavidin, the potential for interaction was between four streptavidin tetramers and one conjugate molecule (Fig. 3). The SPR curves for the binding to the streptavidin-deposited surface are shown in Fig. 4. The injection of the conjugate over the streptavidin-deposited surface increased the response when increasing the concentration from 1 to 50 nM. However, such an increase in the response was not observed when a biotin-precoated streptavidin surface (injected biotin conc.: 1 mM) was used. These results indicate that the biotin in the conjugate is actually recognized by streptavidin.

The binding curves in Fig. 4 can be expressed by the following equation (1), assuming the first order equation:

$$R_t = (R_{eq} - R_0)\ [\ 1\text{-}\exp(\text{-}k_{on}t)]] + R_0 \qquad (1)$$

whrere $R_t$ is the response at time $t$, $R_0$ the initial response, and $k_{on}$ the pseudo-first order rate for the binding. The binding rate constant ($k_{bind}$), dissociation rate constant ($k_{diss}$), and association equilibrium constant ($K_a$) were all calculated using the following equations (2):

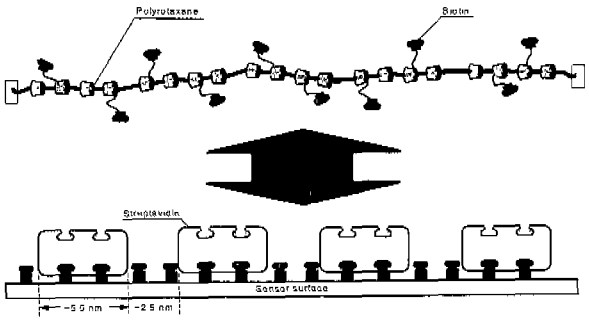



**Fig. 3**. Schematic image of streptavidin recognition of biotin molecule in polyrotaxane-biotin conjugate on biotin-immobilized IAsys sensor surface.

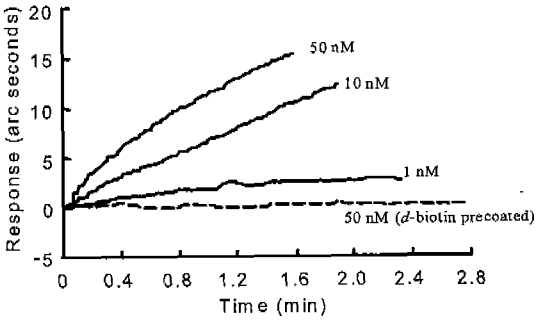



**Fig. 4**. SPR curves for polyrotaxane-biotin conjugate binding to streptavidin-deposited surface. The concentration was calculated on a biotin basis.

$$k_{on} = k_{bind}\ [\text{biotin}] + k_{diss},\ K_a = k_{bind} / k_{diss} \qquad (2)$$

where [biotin] is the concentration of biotin in the conjugate. Fig. 5 shows a plot of $k_{on}$ as a function of the concentration of biotin in the conjugate. The resulting plot was fitted to a straight line ($r^2 = 0.99$) using the linear least-square method. Thus, $k_{bind}$ and $k_{diss}$ were calculated using equation (2). The $k_{bind}$ and $k_{diss}$ values of the conjugate were determined to be $1.3 \times 10^5$ M$^{-1}$sec$^{-1}$ and $1.6 \times 10^{-3}$ sec$^{-1}$, and the resulting $K_a$ value was $8.1 \times 10^7$ M$^{-1}$. According to the report of Green [25], the values of $k_{bind}$, $k_{diss}$, and $K_a$, as determined by $^{14}$C-labeled biotin binding in a solution state, are $6.9 \times 10^7$ M$^{-1}$sec$^{-1}$, $9.0 \times 10^{-8}$ sec$^{-1}$, and $7.6 \times 10^{14}$ M$^{-1}$, respectively. Accordingly, the above results indicate that the binding strength between streptavidin and biotin decreased due to conjugation with the polyrotaxane scaffolds. Several factors that affect the binding features were considered: i) differences in analytical methods, ii) orientation of ligand (biotin), iii) polymer chain length, and iv) density of ligand in the conjugate or receptor (streptavidin). It is known that the kinetics of multivalent interactions lead to large differences between an assay in a solution and an SPR analysis [10]. For example, the method of $^{14}$C-labeled biotin binding in a solution state is conducted over several hours [25]. In contrast, in an SPR analysis, the kinetic data can be obtained within se-

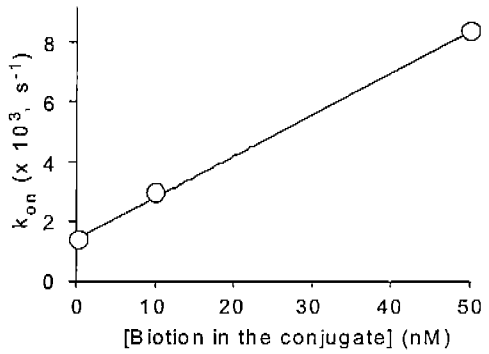



**Fig. 5.** $k_{on}$ values calculated using sensorgrams in Fig. 4 as a function of biotin concentration in polyrotaxane-biotin conjugate.

conds to minutes. Although an attempt was made to measure the binding property of *d*-biotin using SPR, this was impossible because of the limited ability to detect binding with the low molecular weight ligands [10]. Thus, it is difficult to directly compare the kinetic parameters of the *d*-biotin and conjugate obtained in the current experiment. However, the decreased $K_d$ value in comparison with that of *d*-biotin with a $1/10^7$ order would appear to show a reduced affinity with streptavidin because the difference in the analytical methods of an SPR and a solution assay causes a different sensitivity below 100 orders [10]. As for the orientation of biotin, the association of the biotin conjugated with the polyrotaxane should be taken into account. When the concentration of the conjugate increased over 1 wt%, the conjugate was found to be aggregated. The conjugate was soluble in the buffer used in this analysis below 1 wt%, yet the time taken to complete the dissolution of the conjugate was much longer than that for hydroxycarbamoyl-polyrotaxane (data not shown). This result suggests that association of the conjugate may exist under SPR conditions, which may be one of the factors for the reduced $K_d$ value. The polymer chain length may be related to the valency [9,14]. As mentioned above, one conjugate molecule can potentially interact with four streptavidin molecules (Fig. 3), thus, the chain length may not be a serious factor. If this factor is negligible, the steric fitting between the biotin in the conjugate and streptavidin should be considered, which may be related to the density of the biotin in the conjugate or the streptavidin on the surface. In the experimental results, one biotin molecule was introduced to every two α-CD molecules, and the biotin-immobilized α-CDs threading onto the PEO chain may have been diffusible along with the PEO chain. Based on the supramolecular characteristic, the density of the biotin in the conjugate may have been changeable to some extent. Kiessling *et al.* reported that ligand residue density in multivalent polymers is one of the key factors in controlling multivalent binding [34]. Furthermore, Kahne *et al.* proposed that changing the surface density of carbohydrate ligands is the regulatory pathway for binding proteins [35]. These phenomena have been proved by both SPR analysis and thermodynamic analysis [10]. Therefore, when taking these reports into account, various biotin-polyrotaxane conjugates with different densities should be synthesized and analyzed by SPR and alternative analytical methods, such as isothermal titration microcalorimetry and enzyme-linked lectin assays [36, 37], to clarify the multivalent effect.

Recently, we have tried to develop the multivalent ligands of the polyrotaxanes as specific inhibitor of intestinal peptide transporter, PepT1 that transports di- and tripeptides for the intake of proteins [38]. In that research, a transportable dipeptide, valyl-lysine, was conjugated with the polyrotaxane, and the effect of the supramolecular structure of the polyrotaxane on the multivalent interaction was clarified. Based on this approach, it is expected that polyrotaxane-biotin conjugates interact specifically with sodium dependent multivitamin transporter (SMVT) that can transport biotin in intestine and brain [39, 40]. If the polyrotaxane is conjugated with biotin and peptide drugs, this conjugate may be applicable for drug targeting to SMVT.

## CONCLUSION

It was preliminarily demonstrated that biotin as a model ligand in a polyrotaxane-biotin conjugate was recognized by a streptavidin-deposited sensor surface when using the SPR technique. Biotin was immobilized into hydroxyl groups of α-CDs in the polyrotaxane without any contamination. Under the SPR analytical conditions, there was a potential for an interaction between four streptavidin tetramers and one molecule of the conjugate. It would appear that the synthesis of polyrotaxane-biotin conjugates with different biotin densities and an alternative analysis to SPR are necessary to clarify the detailed interaction features. A study on the effect of ligand density and an alternative analysis is currently in progress.

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