Tissue-Targeting Ability of Saccharide–Poly(L-Lysine) Conjugates

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To evaluate the effect of introducing a saccharide moiety to poly(amino acids) on tissue distribution, several glycoconjugates of ε-(2-methoxyethoxyacetyl)-poly(L-lysine) of three molecular weights were synthesized using an octylene spacer between the sugar and polymer chain. Methoxyethoxyacetylation of the ε-amino group of the lysine unit in poly(L-lysine) was useful for avoiding nonspecific distribution to many tissues as the result of cationic charges. The tissue-targeting ability of each saccharide moiety was considered as the actual amount changed in each tissue caused by saccharide modification. Galactose terminated saccharides such as galactose, lactose and *N*-acetylgalactosamine accumulated exclusively in the liver, probably by the hepatic receptor. These conjugates could therefore be good carriers for a drug delivery system to the liver. On the other hand, the mannosyl and fucosyl conjugates were preferentially delivered to the reticuloendothelial systems such as those in the liver, spleen and bone marrow. In particular, fucosyl conjugates accumulated more in the bone marrow than in the spleen. Xylosyl conjugates accumulated mostly in the liver and lung. Generally, the accumulated amount in the target tissue increased with increasing molecular weight and an increased number of saccharides on one molecule of polymer.

Keywords tissue targeting; poly(L-lysine); glycoconjugate; tissue distribution; drug carrier; drug delivery system

The carbohydrate moieties of glycoproteins play an important role in tissue distribution to the targeted organ in the body. The goal of this project is to find a synthetic glycoconjugate which would be distributed to specific organs as a result of biological recognition between carbohydrate chains and receptors. So far, many groups have reported that once a carbohydrate has been conjugated to certain types of carriers such as proteins,[2] polysaccharides[3] and synthetic polymers,[4] the tissue distribution of the carriers are changed. Many investigators used only a few kinds of carbohydrates, such as galactose and mannose, because their aim was to target drug delivery to the liver. Among them, Kojima's group systematically studied the tissue distribution of many glycoconjugates of bovine serum albumin (BSA) using several types of spacers.[5] Considering further application to humans however, BSA is undesirable because of its antigenicity. If the plasma clearance of a carrier occurs too late, it would accumulate in other undesired organs. We selected poly(L-amino acids) with various molecular weights which should have different plasma clearance rates.

Poly(L-lysine) is a well-known polycationic macromolecule which is non-antigenic and biodegradable. Poly(L-lysine) has been studied as a potential drug carrier for cancer therapy.[6] However, it tends to bind non-specifically to the surface of all mammalian cells and is toxic, presumably due to its polycationic properties.[7] Therefore, it would be necessary to decrease the cationic charges by acylating the free amino groups of poly(L-lysine) in order to apply it as a carrier for a drug delivery system.

We previously synthesized glycoconjugates of human serum albumin[8] and poly(L-glutamic acid),[9] then initially studied tissue distribution and cell binding affinity using these synthetic glycoconjugates to optimize the spacer length (number of methylenes) between the carbohydrate ring and the carrier.[10] In this study, we prepared several glycoconjugates of poly(L-lysine) with an octylene (C8 carbon–chain) spacer, and estimated the tissue-targeting ability of these synthetic glycoconjugates in normal rats based upon changes in the tissue concentration. The effect that introducing a saccharide moiety had on the tissue distribution of the carrier is discussed.

MATERIALS AND METHODS

Optical rotation was measured in water at room temperature with a Perkin-Elmer Model 141 polarimeter (Perkin-Elmer Corp., Norwalk, U.S.A.). IR spectra were recorded with a Hitachi 215 spectrometer (Hitachi, Tokyo, Japan). Dialysis was performed with Spectra/Por 3 (molecular weight cutoff 3500) purchased from Spectrum, Houston, U.S.A., against purified water at 4 °C. The borate buffer (pH 9.1) consisted of 0.08 M sodium tetraborate containing 0.35 M potassium hydrogen carbonate.

Animals and Chemicals Male SD rats (6—7 weeks old) weighing 200—230 g were purchased from Sankyo Labo Service Co., Ltd. (Tokyo, Japan). Animals were maintained on standard rat foods and water *ad libitum*. Poly(L-lysine) hydrobromide of three molecular weights, namely, 11700 [DP (degree of polymerization) 56], 21700 (DP 104), and 59000 (DP 282), were purchased from Sigma Chemical Company (St. Louis, U.S.A.). The *N*-succinimide ester of 3-(4-hydroxyphenyl)propanoic acid was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Na[^{125}I] was purchased from Amersham Japan (Tokyo, Japan). All other chemicals were from commercial sources.

Synthesis of [8-(β-D-Galactopyranosyloxy)octylcarbonyl]-ε-[3-(4-hydroxyphenyl)propanoly]-ε-(2-methoxyethoxyacetyl)-poly(L-lysine); [PLL-(Gal)9] A solution of 8-hydrazinocarbonyloctyl β-D-galactopyranoside (**2a**, 35 mg, 0.10 mmol),[8] which was synthesized from methyl 9-(β-D-galactopyranosyloxy)nonanate (**1a**) in dry *N*,*N*-dimethylformamide (DMF, 1 ml) was cooled to −20 °C, then 4 N HCl–dioxane (0.15 ml) and *tert*-butyl nitrite

(18 mg, 0.17 mmol) in dry DMF (0.1 ml) were added. The mixture was stirred for 30 min to give an active acid azide solution, which was added to the aqueous solution of poly(L-lysine) HBr salt **3** (30 mg, 2.56 mmol) dissolved in the borate buffer (6 ml). A solution of 3-(4-hydroxyphenyl)-propanoic acid *N*-succinimide ester (6.7 mg, 25.4 mmol) in dry DMF (0.1 ml) was added to the aqueous solution, and the mixture was stirred at 4 °C in a cooling chamber for 18 h. After the addition of 47% hydrobromic acid to pH 0.85, the mixture was dialysed. The dialysate was lyophilized to give the intermediate conjugate **6** (46.7 mg) as a colorless solid. This was used for the next step without further purification.

A mixture of 2-methoxyethoxyacetic acid (360 mg, 2.69 mmol), *N*-hydroxysuccinimide (320 mg, 2.78 mmol) and 1,3-dicyclohexylcarbodiimide (530 mg, 2.78 mmol) in dry tetrahydrofuran (3 ml) was stirred for 18 h at 4 °C, then the reaction mixture was filtered. The filtrate was concentrated to obtain 2-methoxyethoxyacetic acid *N*-succinimide ester (2.69 mmol). To the intermediate conjugate **6** (75 mg, 6.14 mmol) in water (10 ml), borate buffer (10 ml) and a DMF solution (2 ml) of 2-methoxyethoxyacetic acid *N*-succinimide ester (2.63 mmol) were added. The mixture was stirred at 4 °C for 18 h. After removing the insoluble material by centrifugation, the supernatant was purified by column chromatography on Sephadex G-100 (Pharmacia LKB Biotechnology, Tokyo, Japan). The higher molecular weight fraction was dialyzed and then lyophilized to give PLL–(Gal)9 (40.5 mg) as a colorless solid. The other glycoconjugates shown in Table I were also synthesized by the same procedures.

The number of combined saccharide moieties to 1 mol of each conjugate (N_{sac}) was determined by the phenol–sulfuric acid method.[11] The number of 3-(4-hydroxyphenyl)propanoyl moieties in 1 mol of each conjugate (N_{HPP}) was determined by quantitative ultraviolet absorption at 276 nm. The number of uncombined amino groups in 1 mol of each conjugate (N_{amino}) was determined using fluorescamine.[12] Then, the number of 2-methoxyethoxyacetyl combined ε-amino groups (N_{MEA}) was calculated by the following equation:

$$N_{MEA} = DP - (N_{amino} + N_{sac} + N_{HPP}) \quad (1)$$

Synthesis of ε-[3-(4-Hydroxyphenyl)propanoyl]-poly(L-lysine) HBr Salt; [PL·HBr] To a solution of poly(L-lysine) HBr salt **3** (75 mg, 6.14 mmol) in the borate buffer (10 ml) was added a solution of 3-(4-hydroxyphenyl)propanoic acid *N*-succinimide ester (8.4 mg, 31.9 mmol) in dry DMF (0.2 ml) at 4 °C, and the mixture was stirred at 4 °C for 18 h. After adding 47% hydrobromic acid to attain pH 0.85, the resulting solution was dialyzed and lyophilized to give the conjugate PL·HBr (143.8 mg) as a colorless solid.

PL·HBr: IR(KBr): 3400, 3300, 1650, 1540 cm^{-1}, $[\alpha]_D$ −47.3° (c=0.51, H_2O), N_{HPP}=3.

Synthesis of ε-[3-(4-Hydroxyphenyl)propanoyl]-ε-(2-methoxyethoxyacetyl)-poly(L-lysine); [PLL] To a solution of poly(L-lysine) HBr salt **3** (75 mg, 6.14 mmol) in the borate buffer (10 ml) was added 3-(4-hydroxyphenyl)propanoic acid *N*-succinimide ester (8.4 mg, 31.9 mmol) in dry DMF (0.2 ml) and a DMF solution (2 ml) of 2-methoxyethoxyacetic acid *N*-succinimide ester (2.63 mmol), then the mixture was stirred at 4 °C for 18 h. The reaction mixture was purified by dialysis and subsequent lyophilization to give the conjugate PLL (40.5 mg) as a colorless solid. PLM and PLH having another molecular weight were also derived from **4** and **5** respectively by the same procedures as described above.

Radioiodination of Poly(L-Lysine) Derivatives The 3-(4-hydroxyphenyl)propanoyl moieties of the polymer were radiolabeled using the chloramine T method.[13] Briefly, 20 μl of aqueous polymer solution (2.5 mg/ml) was mixed with 50 μl of 0.5 M phosphate buffer (pH 7.4), 0.3 mCi of [^{125}I]iodine and 10 μl of chloramine T (2 mg/ml), then vigorously mixed for 30 s at room temperature. Thereafter, 50 μl of sodium pyrrosulfite (2.5 mg/ml) and 10 μl of potassium iodide (100 mg/ml) were added to terminate the reaction. Excess [^{125}I]iodine was removed by gel filtration on a Sephadex G-25 column (Pharmacia LKB Technology, Tokyo, Japan) with isotonic sodium chloride solution. Two milliliters of the polymer fraction were collected. For animal experiments, injection samples were prepared by adjustment with 5% BSA and unlabeled polymer to give a final concentration of 1% BSA and 1 mg/ml of polymer, respectively. The final specific activity was about 20 μCi/mg of polymer.

Degradability of [^{125}I]Polymer in Liver Homogenate and Plasma One gram of liver excised from a SD rat was homogenized in 3 ml of phosphate buffered saline (pH 7.4) with the Polytron homogenizer (KINEMATICA GmbH LITTAU, Switzerland). After centrifugation at 1500 × *g* for 5 min, the supernatant was collected and used as the tissue homogenate. Fifty microliters of [^{125}I]polymer (1 mg/ml) were added to 0.45 ml of liver homogenate and incubated at 37 °C. At various intervals, 50 μl samples were withdrawn from the mixture and placed into 0.4 ml of 15% trichloroacetic acid (TCA) together with 0.1 ml of 5% BSA. Acetonitrile was used for PL·HBr instead of 15% TCA. After centrifugation, the radioactivity in the precipitate and supernatant, which were regarded as the polymer and low molecular weight fractions respectively, were separately measured in a γ-scintillation counter (ARC-301B, Aloka, Tokyo, Japan). Degradability in plasma taken from the same animal was also examined in the same manner.

Tissue Distribution of [^{125}I]Polymer in Rats Male SD rats anesthetized with diethyl ether were intravenously injected (1 mg/kg body weight) with ^{125}I-labeled polymer *via* the femoral vein. Blood samples (200 μl) were taken from the jugular vein at 0.5, 1, 2 and 3 min after the injection, and centrifuged to separate the plasma. Five minutes after the administration, animals were sacrificed by bleeding from the abdominal aorta, then the major tissues were excised and weighed. The radioactivity levels in all the specimens were measured and the tissue concentrations were calculated as the percentage of dose per g of tissue. The tissue distribution at 60 min after the injection was also investigated in the same manner. Each group consisted of 3 animals in all the experiments.

Evaluation of the Tissue-Targeting Ability The tissue-targeting ability, or preferential accessibility to a particular tissue of each glycoconjugate, was evaluated as the

increased tissue concentration by the effect of a specific saccharide chain being modified on the carrier. The targeting ability was thus calculated using the following equation:

$$C_{t,sub} = C_{t,modified} - C_{t,carrier} \quad (2)$$

where, $C_{t,modified}$ and $C_{t,carrier}$ are the tissue concentrations (% of dose/g tissue) of injected polymers with modified saccharides and the unaltered carrier, respectively. $C_{t,sub}$ (subtracted tissue concentration) is the tissue-targeting ability.

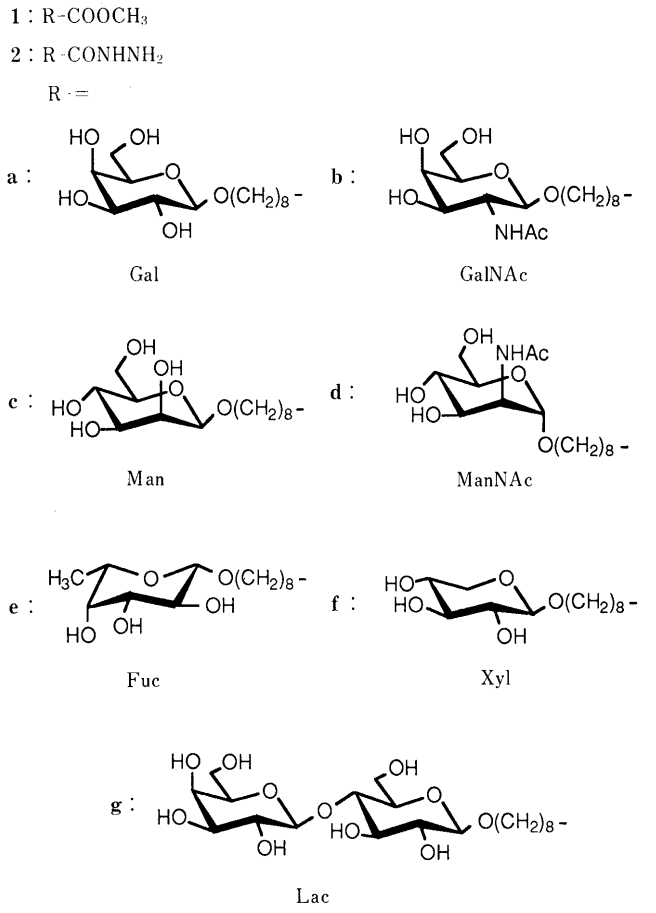

Fig. 1. Structures of Methyl 9-Glycosyloxynonanates **1** and 8-Hydrazinocarbonyloctyl Glycosides **2** and Abbreviations for the Sugar Components

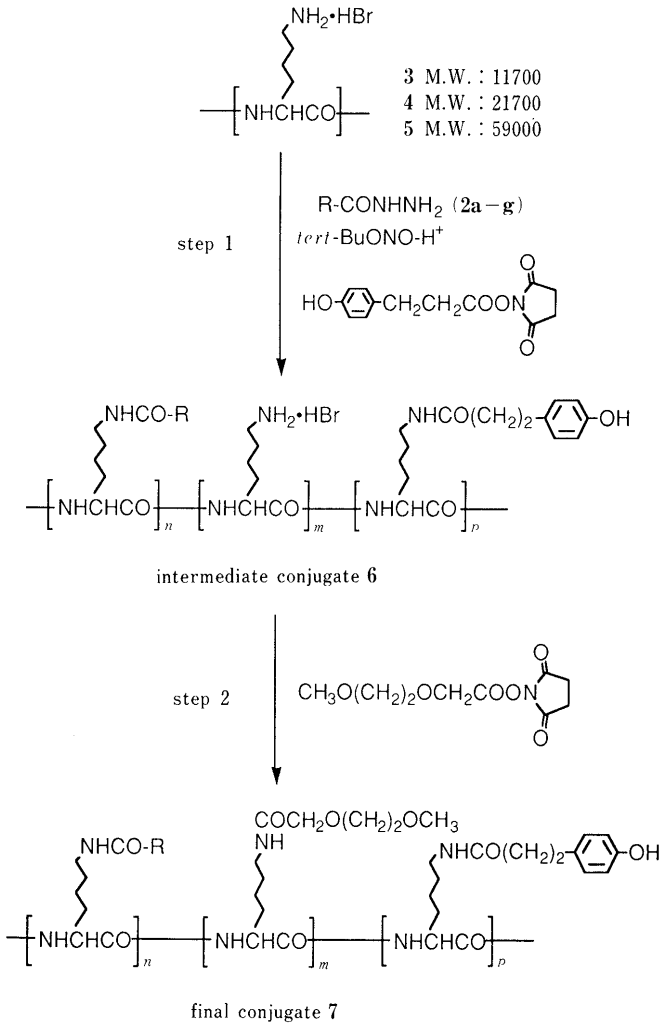

Chart 1. Synthesis of Saccharide–Poly (L-lysine) Conjugates
R is shown in Fig. 1.

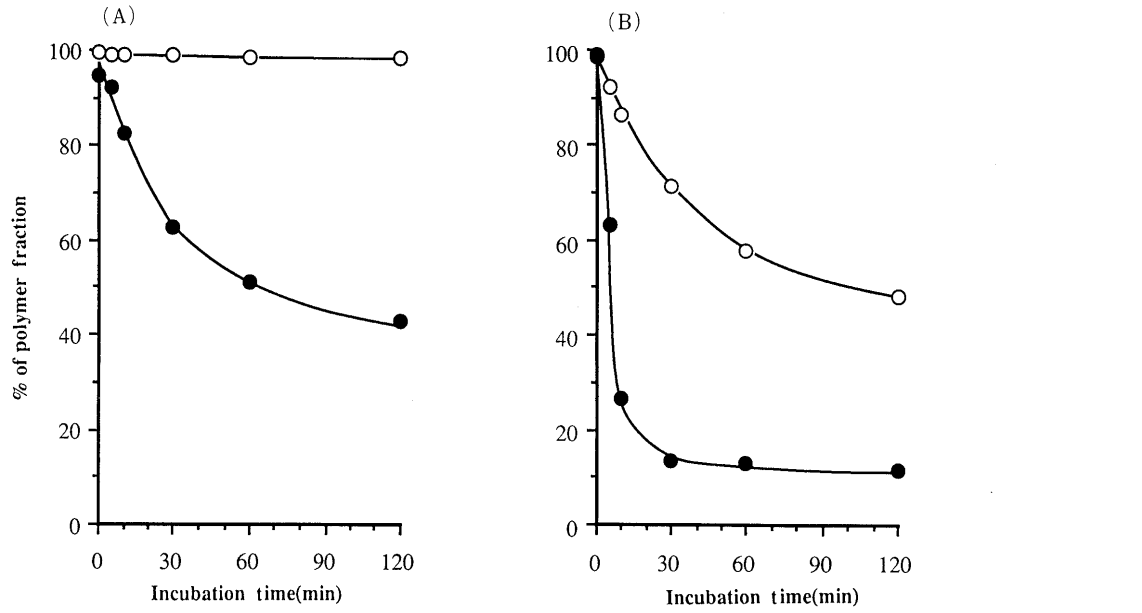

Fig. 2. Degradation of PL·HBr (●) and PLL (○) after Incubation at 37°C in Plasma (A) and Liver Homogenate (B)

RESULTS AND DISCUSSION

Synthesis of Poly(L-Lysine) Glycoconjugates We attempted to synthesize saccharide–poly(L-lysine) conjugates by coupling the ε-amino groups of three poly(L-lysine) with several types of saccharides. Preparation of methyl 9-glycosyloxynonanates **1a**—**g** has already been reported,[8,14] and these structures are shown in Fig. 1 together with the abbreviations for the saccharides. Treatment of **1a**—**g** with hydrazine monohydrate gave the corresponding hydrazides **2a**—**g**, which were further derived to a reactive acid azide with *tert*-butyl nitrite. The three types of poly(L-lysine) were acylated in a buffer (pH 9.1) containing an acid azide and 3-(4-hydroxyphenyl)propionic acid *N*-succinimide ester. This 3-(4-hydroxyphenyl)propanoyl moiety is necessary for later radioiodination. Lyophilization of the dialysate gave a saccharide-poly(L-lysine) conjugate (intermediate conjugate **6** in Chart 1) as a colorless solid with all the saccharides (Chart 1, step 1). The number of combined saccharide moieties to 1 mol of each conjugate (N_{sac}) and the number of 3-(4-hydroxyphenyl)propanoyl moieties to 1 mol of each conjugate (N_{HPP}) were measured.

We then acetylated the free amino groups, which are the unsubstituted ε-amino groups of lysine residues in the intermediate conjugate, in order to decrease its cationic charges. However, only an insoluble fraction was obtained. We thus selected 2-methoxyethoxyacetic acid[15] as the acylation agent. When intermediate conjugates were acylated by 2-methoxyethoxyacetic acid *N*-succinimide ester (Chart 1, step 2), the resulting acylated conjugates (final conjugate **7** in Chart 1) were successfully soluble in water. The numbers of both the unsubstituted and 2-methoxyethoxyacetyl combined ε-amino groups (N_{amino} and N_{MEA} respectively) were determined. Table I lists N_{sac}, N_{HPP}, N_{amino}, N_{MEA}, and the physical properties, together with the abbreviations of the final conjugates.

We also synthesized [3-(4-hydroxyphenyl)propanoyl]-poly(L-lysine) conjugate (PL·HBr derived from **3**) and three (2-methoxyethoxyacetyl)-[3-(4-hydroxyphenyl)propanoyl]-poly(L-lysine) conjugates, namely PLL (derived from **3**), PLM (derived from **4**) and PLH (derived from

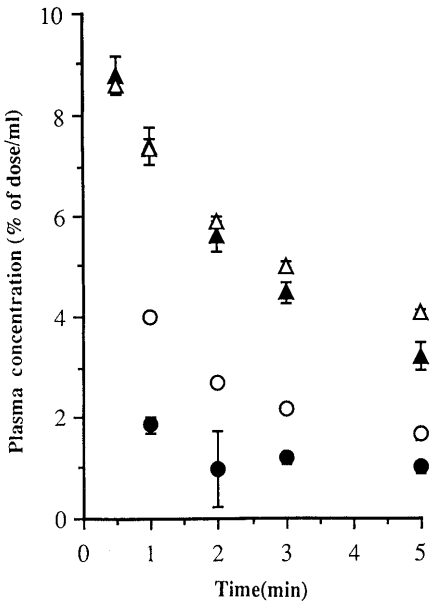

Fig. 3. Plasma Concentration of Several Poly(L-Lysine) Derivatives in Rats after 1 mg/kg i.v. Injection

Each point represents the mean ± S.D. ($n=3$). In PLL and PL·HBr, the point at 0.5 min was not determined. Δ, PLH; ▲, PLM; ○, PLL; ●, PL·HBr.

TABLE I. Synthesis of Saccharide–Poly(L-Lysine) Conjugates and Their Properties

Sac[a]	PL[b]	Conjugate[c] abbreviation	N_{sac}	N_{HPP}	N_{amino}	N_{MEA}	$[\alpha]_D$ (c)[d]	IR (KBr) cm^{-1}
—	3	PLL	0	4	4	48	−47.3 (0.51)	3400, 3300, 1650, 1540
1a	3	PLL–(Gal)9	9	6	4	37	−18.2 (0.52)	3300, 1650, 1540
1b	3	PLL–(GalNAc)18	18	6	2	30	−10.7 (0.41)	3300, 1650, 1545, 1110, 1080
1c	3	PLL–(Man)12	12	5	1	38	−27.7 (0.52)	3300, 1650, 1540
1d	3	PLL–(ManNAc)22	22	3	0	31	+0.9 (0.45)	3400, 1655, 1545, 1155, 1080
1e	3	PLL–(Fuc)22	22	6	8	20	−5.1 (0.54)	3300, 2930, 1650, 1550, 1100
1f	3	PLL–(Xyl)10	10	4	9	33	−5.3 (0.57)	3355, 1655, 1545, 1100
1g	3	PLL–(Lac)20	20	4	9	23	−21.8 (0.27)	3350, 1655, 1540, 1100, 1040
—	4	PLM	0	6	6	92	−70.0 (0.51)	3400, 2935, 1655, 1545
1a	4	PLM–(Gal)43	43	6	17	38	−9.6 (0.25)	3400, 3300, 1655, 1545, 1080
1b	4	PLM–(GalNAc)43	43	4	6	51	−5.8 (0.52)	3460, 3300, 1650, 1545, 1080
1c	4	PLM–(Man)43	43	4	29	28	−23.0 (0.50)	3300, 1650, 1545
1d	4	PLM–(ManNAc)30	30	3	0	71	+6.9 (0.44)	3304, 1655, 1515, 1130, 1075
1e	4	PLM–(Fuc)43	43	6	2	53	−5.1 (0.54)	3360, 1650, 1540, 1070
1f	4	PLM–(Xyl)29	29	3	10	62	−7.3 (0.55)	3400, 1655, 1545, 1135, 1065
1g	4	PLM–(Lac)53	53	7	5	39	−5.9 (0.56)	3300, 1650, 1545, 1080, 1050
—	5	PLH	0	6	27	249	−18.4 (0.51)	3400, 3330, 1650, 1550
1a	5	PLH–(Gal)93	93	20	45	124	−10.4 (0.55)	3300, 1660, 1545, 1140, 1090
1b	5	PLH–(GalNAc)37	37	8	22	215	−6.9 (0.48)	3300, 1650, 1540, 1140, 1050
1c	5	PLH–(Man)100	100	9	106	67	−17.7 (0.48)	3420, 1650, 1545, 1140, 1090
1d	5	PLH–(ManNAc)53	53	7	2	220	−2.8 (0.29)	3380, 1660, 1545, 1115
1e	5	PLH–(Fuc)92	92	13	4	173	−6.1 (0.64)	3300, 2930, 1650, 1550, 1100

a) Starting hydrazides used for synthesis. Structures are shown in Fig. 1. *b*) Starting poly (L-lysine) hydrogenbromide used for synthesis. *c*) Number following parentheses represents the number of each sugar moiety on one mole of carrier. *d*) Optical rotations were measured in water at room temperature.

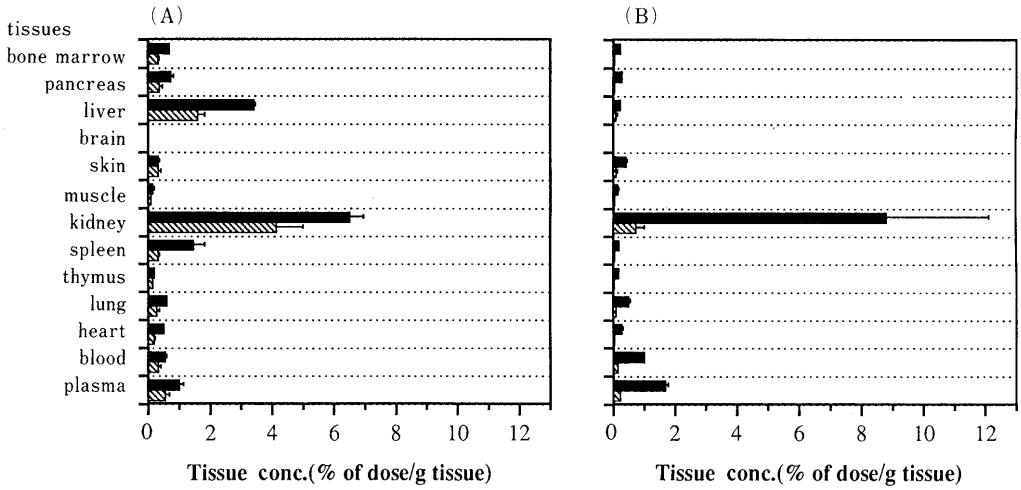

Fig. 4. Tissue Concentrations of PL·HBr (A) and PLL (B) in Rats 5 min (Solid Bar) and 60 min (Hatched Bar) after 1 mg/kg i.v. Injection
Each point represents the mean ± S.D. ($n=3$).

5), by procedures similar to those described above. These were used as control carriers in the following animal experiments.

All the samples were radioiodinated by the chloramine T method and used within one week.

Evaluation of Methoxyethoxyacetyl–Poly(L-Lysine) as the Carrier First, we evaluated the usefulness of ε-[2-methoxyethoxyacetyl]-poly(L-lysine) as the carrier. In Fig. 2, the stability of both PL·HBr and PLL *in vitro* are shown. PL·HBr was rapidly degraded to low molecules during incubation in plasma and liver homogenates at 37 °C. By contrast, PLL was slowly degraded by the liver enzymes and was completely stable against the plasma enzymes. This suggests that PLL retained moderate biodegradability. The plasma concentration–time and the tissue distribution profiles at 5 and 60 min after the intravenous administration of PL·HBr or PLL are shown in Figs. 3 and 4, respectively. PL·HBr was rapidly cleared from the plasma and broadly accumulated in various tissues, especially in the kidney and liver. Furthermore, 60 min after the injection it still remained in many tissues. It is assumed that highly positive charged PL·HBr reacted electrostatically with many tissues with negatively charged constitutents. Sela and Katchalski had mentioned the same assumption in their review articles.[7] On the other hand, PLL was more slowly cleared from the plasma than PL·HBr, and accumulated exclusively in the kidney 5 min after the injection, then almost cleared from the whole body by 60 min after the injection. This rapid body clearance of PLL might be due to both a decreased tissue uptake and a greatly increased urinary excretion caused by the methoxyethoxyacetylation of ε-amino residues. Therefore, ε-(2-methoxyethoxyacetyl)-poly(L-lysine) could be a good carrier for evaluating an increased concentration by glycoconjugation in each tissue, except in the kidney.

The plasma concentration–time profiles of PLM and PLH are also shown in Fig. 3. The rate of plasma clearance decreased with increasing molecular weight.

Evaluation of the Tissue-Targeting Ability of Glycoconjugate There are several means of evaluating the targeting ability of various materials to a particular tissue *in vivo*. These include the tissue-plasma partition coefficient, tissue distribution clearance, tissue concentration, and others. Since our concern was the delivery amount to the target tissue using a drug-carrier system, we decided to compare the tissue concentration. This was calculated using the subtracted tissue concentration ($C_{t,sub}$) which is obtained by subtracting the tissue concentration after the administration of the original carrier from that of glycosyl carrier, as shown in Eq. 2. Therefore, if the $C_{t,sub}$ of a certain tissue was positive, targeting to this tissue by the modified saccharide chain would be possible. Once we know the magnitude of $C_{t,sub}$, we will be able to estimate the delivery amount to the target tissue caused by the modification of a carrier with a saccharide.

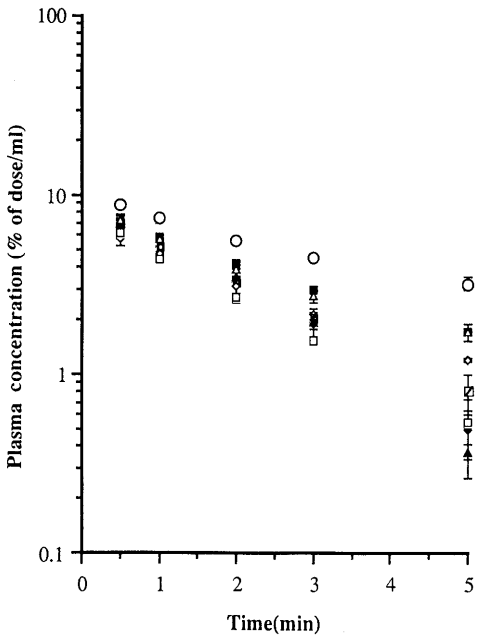

Fig. 5. Plasma Concentration of Several PLM Glycoconjugates in Rats after 1 mg/kg i.v. Injection
Each point represents the mean ± S.D. ($n=3$). ○, PLM; ◆, PLM–(Gal) 43; ▨, PLM–(GalNAc) 43; ◇, PLM–(Man) 43; ■, PLM–(ManNAc) 30; □, PLM–(Fuc)-43; ▲, PLM–(Lac) 53; △, PLM–(Xyl) 29.

Figure 5 shows typical results of the plasma concentration–time profiles after the intravenous administration of PLM and its glycosyl derivatives. All the glycosyl PLM

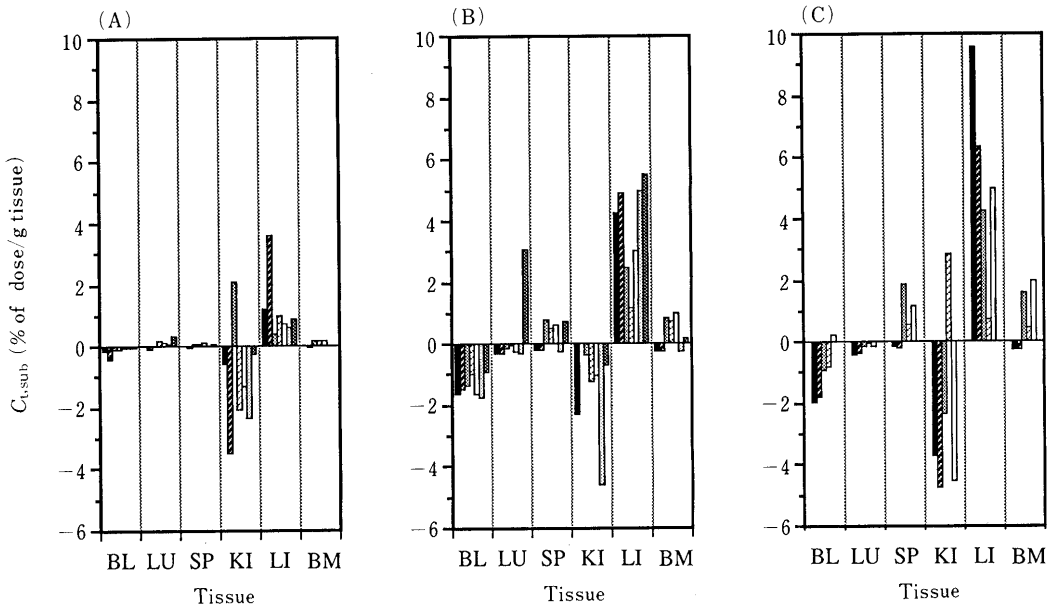

Fig. 6. Subtracted Tissue Concentrations ($C_{t,sub}$) of Glycoconjugates of PLL (A), PLM (B) and PLH (C) in Six Tissues

$C_{t,sub}$ was calculated from data in Table II. Column symbol: ■, Gal; ▨, GalNAc; ▩, Man; ▨, ManNAc; □, Lac; □, Fuc; ▩, Xyl. Tissue abbreviation: BL, blood; LU, lung; SP, spleen; KI, kidney; LI, liver; BM, bone marrow.

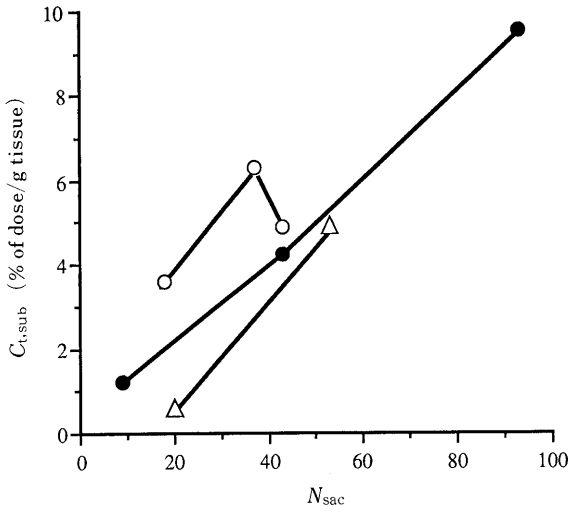

Fig. 7. Subtracted Tissue Concentrations ($C_{t,sub}$)–Number of Combined Saccharide Moieties to 1 mol of Each Conjugate (N_{sac}) Relationship in the Liver by Carriers Modified with Gal (●), GalNAc (○) and Lac (△)

were more rapidly cleared from the blood stream than the unaltered PLM. The same results were obtained using PLL and PLH (data not shown). These rapid plasma clearances may be attributed to the increased uptake by some tissues.

Next, concentrations in 12 tissues 5 min after the administration of each conjugate were measured. In the heart, thymus, muscle, skin, brain and pancreas, the measured tissue concentrations were quite small, and the calculated $C_{t,sub}$ of these tissues were nearly zero (data not shown). Accordingly, we concluded that targeting to these tissues would be impossible with the methodology described here (modification with saccharide). The measured concentrations of six other tissues are listed in Table II and are grouped according to their molecular weight.

Figure 6 shows the $C_{t,sub}$ of the other six tissues. The results for each molecular weight seem to follow a similar pattern. In the blood and kidney, $C_{t,sub}$ was highly negative. This suggests that all the glycosyl carriers were rapidly cleared from the blood, but slowly from the whole body. By contrast, the $C_{t,sub}$ of the reticuloendothelial system (RES) were highly positive, and the tissue distribution properties of the glycosyl carriers among these tissues depended on the type of saccharide modified on the carrier. Carriers modified with Gal, GalNAc or Lac accumulated exclusively in the liver, suggesting that they were taken up into the liver by receptor-mediated endocytosis *via* the galactose-recognizing hepatic receptor, which have been wellknown since their discovery in 1974.[16] This result indicates that these glycosyl carriers could be useful in targeting a drug delivery system to the liver. In order to compare the magnitudes of $C_{t,sub}$ in the liver among the three saccharides, the relationship between the N_{sac} of each conjugate and the values of $C_{t,sub}$ are plotted in Fig. 7. The $C_{t,sub}$ values of galactosyl and lactosyl carriers increased with the degree of saccharide substitution on 1 mol of carrier and the molecular weight of the polymer. However, PLH–(GalNAc)37 had a higher $C_{t,sub}$ than PLM–(GalNAc)43. It seems that $C_{t,sub}$ is influenced not only by N_{sac} but by the molecular weight of the polymer which changes the plasma clearance. The results in Fig. 7 suggest that the order of targeting ability to the liver is GalNAc > Gal > Lac. This order might reflect the affinity of the glycosyl carrier for the hepatic receptor.[17]

Although the carrier modified with Man, ManNAc or Fuc accumulated in three tissues of the RES, the $C_{t,sub}$ of ManNAc was quite low (see Fig. 6). Namely, modification with ManNAc did not largely affect the tissue distribution properties of the carrier. The N_{sac}–$C_{t,sub}$ relationships of the carriers modified with Man and Fuc are shown in Fig. 8. The levels of both conjugates were increased in the liver depending upon the N_{sac} of the conjugates. Since Kupffer cells and macrophages have mannose receptors,[18] the

TABLE II. Tissue Concentrations of Saccharide–Poly(L-Lysine) Conjugates in Rats 5 min after 1 mg/kg i.v. Injection

PL	Conjugate abbreviation	Tissue concentration 5 min after administration (% of dose/g tissue)[a]					
		Blood	Lung	Spleen	Kidney	Liver	Bone marrow
3	PLL	0.979±0.031	0.520±0.021	0.176±0.013	8.805±3.317	0.216±0.007	0.211±0.011
	PLL–(Gal)9	0.842±0.102	0.538±0.071	0.163±0.012	8.227±3.028	1.443±0.137	0.212±0.022
	PLL–(GalNAc)18	0.525±0.054	0.396±0.067	0.130±0.011	5.328±1.695	3.806±0.626	0.154±0.005
	PLL–(Man)12	0.867±0.106	0.505±0.053	0.247±0.027	10.873±0.757	0.619±0.018	0.354±0.036
	PLL–(ManNAc)22	0.872±0.076	0.657±0.063	0.251±0.033	6.749±1.854	1.179±0.087	0.357±0.041
	PLL–(Fuc)12	0.925±0.130	0.622±0.044	0.287±0.031	7.491±2.408	0.901±0.017	0.385±0.027
	PLL–(Xyl)10	0.898±0.056	0.837±0.137	0.256±0.016	8.540±2.759	1.068±0.039	0.216±0.032
	PLL–(Lac)20	0.933±0.033	0.554±0.035	0.199±0.017	6.448±0.808	0.830±0.107	0.204±0.021
4	PLM	1.966±0.153	0.577±0.031	0.329±0.018	7.894±1.273	0.545±0.015	0.415±0.040
	PLM–(Gal)43	0.301±0.141	0.224±0.021	0.126±0.041	5.602±4.302	4.792±0.712	0.135±0.056
	PLM–(GalNAc)43	0.489±0.117	0.249±0.009	0.115±0.012	7.902±3.750	5.460±0.207	0.138±0.023
	PLM–(Man)43	0.587±0.047	0.427±0.007	1.097±0.029	7.513±1.589	2.999±0.165	1.246±0.149
	PLM–(ManNAc)30	1.003±0.022	0.544±0.031	0.810±0.039	6.640±3.720	1.701±0.148	1.113±0.054
	PLM–(Fuc)43	0.343±0.030	0.285±0.032	0.914±0.041	6.878±2.767	3.570±0.166	1.372±0.108
	PLM–(Xyl)29	1.028±0.102	3.648±0.410	1.057±0.166	7.203±2.723	6.078±0.735	0.562±0.139
	PLM–(Lac)53	0.223±0.030	0.223±0.008	0.078±0.012	3.284±0.886	5.496±0.498	0.160±0.013
5	PLH	2.373±0.121	0.591±0.029	0.294±0.006	7.152±1.617	0.364±0.007	0.455±0.030
	PLH–(Gal)93	0.400±0.118	0.171±0.031	0.145±0.043	3.431±1.272	9.908±1.148	0.187±0.043
	PLH–(GalNAc)37	0.575±0.202	0.198±0.050	0.102±0.015	2.412±0.796	6.675±0.540	0.163±0.047
	PLH–(Man)100	1.432±0.109	0.449±0.019	2.156±0.357	4.812±0.358	4.603±0.159	2.034±0.096
	PLH–(ManNAc)53	1.559±0.048	0.521±0.068	0.819±0.492	10.015±2.701	1.100±0.173	0.869±0.011
	PLH–(Fuc)92	2.597±0.179	0.413±0.043	1.458±0.144	2.629±0.479	5.337±0.296	2.432±0.030

a) Each value represents mean±S.D. for three animals.

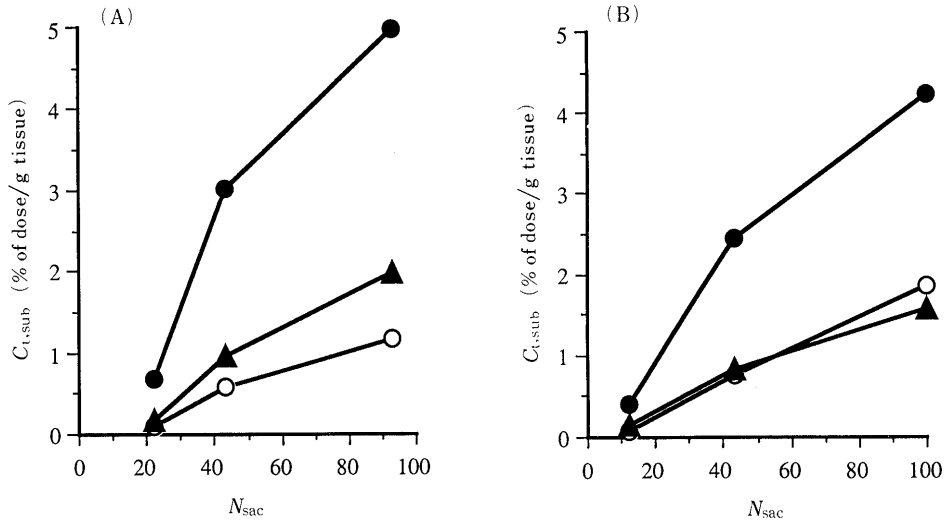

Fig. 8. Subtracted Tissue Concentrations ($C_{t,sub}$)–Number of Combined Saccharide Moieties to 1 mol of Each Conjugate (N_{sac}) Relationship in the RES by Carriers Modified with Fuc (A) and Man (B)

●, in liver; ○, in spleen; ▲, in bone marrow.

mannosyl carrier was presumed to be distributed into the RES. It is noteworthy that the fucosyl carrier accumulated to a greater extent in the bone marrow than in the spleen. The mechanism of this finding remains unknown, however, these results suggest that the modification of carriers with fucose could be useful for preferential drug delivery to the bone marrow.

Lastly, xylosyl carriers, which were derived only from PLL and PLM, accumulated in the liver and lung (see Fig. 9). As the reason for this accumulation in the lung remains unknown, further studies will be performed in our laboratories. Regardless, this indicates that xylosyl carriers could be a targeting device to the lung.

In conclusion, the glycoconjugates shown in this study are believed to have excellent potential as targeting and drug carrying devices to preferential tissues. Moreover, side effects may be preventable since these carriers were quickly cleared from undesirable tissues. The modification described here could be applied to other drug carriers, such as polysaccharides, proteins and synthetic polymers, to develop new drug delivery systems. Currently, investigations are underway to optimize the saccharide content in a carrier and to evaluate the potential of glycosyl carrier-drug conjugates for practical use.

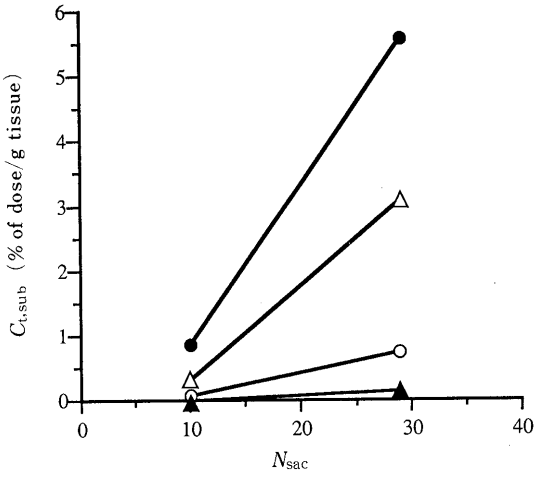

Fig. 9. Subtracted Tissue Concentrations ($C_{t,sub}$)–Number of Xylosyl Moieties to 1 mol of Each Conjugate (N_{sac}) Relationship in the RES and Lung by Carriers Modified with Xylose

●, in liver; ○, in spleen; ▲, in bone marrow; △, in lung.

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