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Intracellular disposition of polysaccharides in rat liver parenchymal and nonparenchymal cells

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Abstract

Binding and internalization of arabinogalactan, pullulan, dextran, and mannan were examined in rat liver parenchymal and nonparenchymal cells using ^{125}I or fluorescein isothiocyanate (FITC) labeled polysaccharides. Binding and uptake of arabinogalactan and pullulan into parenchymal cells was inhibited by asialofetuin, indicating that the asialoglycoprotein receptor is involved in the intracellular disposition of arabinogalactan and pullulan. Uptake of ^{125}I-labeled dextran to parenchymal cells was unchanged upon addition of excess unlabeled dextran, suggesting that dextran uptake occurs via fluid phase endocytosis. Of the polysaccharides tested, mannan showed the strongest specific association with liver nonparenchymal cells. FITC-labeled polysaccharides showed arabinogalactan and pullulan are internalized to liver parenchymal cells, whereas mannan is internalized to nonparenchymal cells. This study demonstrates that intracellular disposition of polysaccharides in the liver occurs via receptor-mediated endocytosis (RME), indicating that RME plays a role in the biodisposition of these polysaccharides as drug carriers.

Keywords: Arabinogalactan; Pullulan; Dextran; Mannan; Hepatotropic drug carrier system; Receptor-mediated endocytosis

1. Introduction

A wide variety of materials have been examined for their ability to deliver drugs to specific sites within the body. Many polysaccharides have structures which are recognized by specific receptors, e.g. asialoglycoprotein receptor, mannose receptor, or scavenger receptors. In general, binding between a receptor and a specific polysaccharide substrate, results in internalization of the polysaccharide into the cell. This sequence of events, binding and internalization, called intracellular disposition, can affect biodisposition of the polysaccharide in vivo.

After intravenous injection, polysaccharide which is not excreted from the kidney distributes to the retic-

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uloendothelial system. The polysaccharides arabinogalactan, pullulan, dextran, and mannan are known to distribute to the liver and can be used as drug carriers. Arabinogalactan is a highly branched, water-soluble polysaccharide, containing numerous terminal galactose residues which are responsible for its binding to the asialoglycoprotein receptor in vitro (Groman et al., 1994). We previously studied the pharmacokinetics and biodisposition of FITC-labeled arabinogalactan in rats and demonstrated its ability to serve as a carrier for the delivery of enzymes and drugs to liver parenchymal cells, via the asialoglycoprotein receptor (Kaneo et al., 2000b). Pullulan is a water-soluble, viscous polysaccharide consisting of three α-1,4-linked glucose molecules, polymerized by α-1,6-linkages to the terminal glucose. We have previously investigated the biodisposition of pullulan in rats and demonstrated that the asialoglycoprotein receptor contributes to the hepatic distribution (Kaneo et al., 2001). Dextran is a polysaccharide consisting of glucose molecules linked through the α-1,6 positions, with various degrees of branching at the α-1,3 positions. It has been shown that FITC-labeled dextran accumulates in the liver and that hepatic uptake of dextran is slow (Kaneo et al., 1997). Mannan consists of an α-1,6-linked mannose backbone, with short 1,3-mannose branches, attached by α-1,2- and α-1,3-linkages. After intravenous injection into mice, mannan is mainly distributed to the liver and spleen (Hojo and Uchiyama, 1972).

Polysaccharides are known to distribute to the liver after intravenous injection, which is desirable for delivery of drugs targeted for the liver. Drug targeting is usually exploited at the cellular level, specific to the action site of the drug, and it has been shown that polysaccharides can deliver drugs to specific cells (Hosseinkhani et al., 2002). One of the more desirable mechanisms for drug delivery into a specific cell is by receptor-mediated endocytosis (RME) because some receptors distribute in the specific cell and recognize specific ligands, which are known to use (Hashida et al., 1997; Kato and Sugiyama, 1997; Tanaka et al., 2004; Wagner et al., 1994). We have previously demonstrated that in vivo, polysaccharides will typically accumulate in the liver, and that different sugar species will target different cell types (Kaneo et al., 2000a). In vivo experiments showed arabinogalactan and pullulan were recognized by the asialoglycoprotein receptor, found in the liver (Kaneo et al., 2000b, 2001), while mannan is recognized by the mannose receptor found in liver nonparenchymal cells. A definitive mechanism for the uptake of dextran to the liver has not yet been established. While dextran has displayed a fluid phase endocytosis ascribed to non-specific receptor binding, some investigators have found that the asialoglycoprotein receptor does play a role in the uptake of dextran to the liver in vivo (Nishikawa et al., 1992).

Little is known about the intracellular disposition of polysaccharides in liver cells. In vivo examination of polysaccharide biodisposition would provide dynamic information regarding the fate of polysaccharides at an organ level, although differentiation between the various polysaccharide-cell interactions (binding and uptake) could be difficult. In this paper, we examined the binding and internalization of arabinogalactan, pullulan, dextran, and mannan into liver parenchymal and nonparenchymal cells, hoping to provide information on the interactions between polysaccharides and liver cells.

2. Materials and methods

2.1. Materials

Arabinogalactan, mannan, asialofetuin (type II, AF), and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, USA). Pullulan was kindly donated by Kuraray Co. Ltd. (Osaka, Japan). Dextran was purchased from Amasiam Pharmacia Biotech (Tokyo, Japan). Collagenase H was obtained from Boehringer Mannheim (Tokyo, Japan). William's medium E was obtained from Dainippon Pharmaceutical Co. Ltd. (Osaka, Japan). Fetal bovine serum (FBS) was purchased from Life Technologies (Gaithersburg, USA) and inactivated at 56 °C for 30 min before use. Fluorescein isothiocyanate (FITC) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals and reagents were of the highest grades commercially available.

2.2. Radio-labeling of polysaccharides

Tyramine derivatives of polysaccharides were prepared as previously described (Kohn and Wilchek, 1984). Polysaccharide (200 mg) was dissolved in 10% acetone in water (4.4 ml). A solution of CNBr (20 mg)

in acetone (0.2 ml) was added at 0 °C. After 2 min, a solution of tetraethylamine (30.4 mg) in acetone (0.2 ml) was added at 0 °C. After a further 2 min, tyramine (40 mg) in 0.1 M Na_2CO_3 containing 0.5 M NaCl (pH 8.5, 4 ml) was added and stirred at 4 °C overnight. The reaction mixture was dialyzed against water, and a macromolecule fraction was lyophilized to yield a white powder containing 1.0–2.1 (w/w%) tyramines. Tyramine derivatives of the polysaccharides (200 μg) were labeled with 0.25 mCi of ^{125}I-iodine (Amersham Biosciences, Tokyo, Japan) by the chloramines T method (Greenwood and Hunter, 1963). Unbound ^{125}I was removed by chromatography on a PD-10 column (Amersham Biosciences).

2.3. FITC-labeling of polysaccharides

FITC-labeled polysaccharides were prepared as previously described (De Belder and Granath, 1973; Kaneo et al., 2000b). The polysaccharide (1 g) was dissolved in methyl sulphoxide (10 ml) containing 3 drops of pyridine. FITC (400 mg) and dibutylin dilaurate (20 mg) were added and the mixture was heated for 2 h at 95 °C. After several precipitations in ethanol, the FITC-labeled polysaccharide fraction was isolated, and purified by size-exclusion chromatography on Sephadex G-25 and then freeze-dried.

2.4. Experimental animals

Wistar strain male rats (6 weeks old) weighting 180–200 g were purchased from Japan SLC, Inc. (Shizuoka, Japan). The rats were kept in cages and maintained on a commercially balanced stock diet (Oriental Yeast Co. Ltd., Tokyo, Japan) with water ad libitum. Before experiments, each rat was anesthetized with pentobarbital. All aspects of the work were performed following the Fukuyama University guidelines for animal experiments.

2.5. Preparation of liver parenchymal cells

Hepatocytes were isolated from male Wistar rats (7 weeks old, Japan SLC, Inc., Shizuoka, Japan) by the collagenase perfusion method (Moldeus et al., 1978). The hepatocytes were centrifuged at 50 × *g* and the resulting pellet washed with Hanks' medium containing 2.4 mM $CaCl_2$ and 10 mM HEPES (pH 7.4). The cells were fractionated on Percoll density gradients to obtain more than 98% viability, as verified by a trypan blue exclusion test. The cells were then resuspended in William's medium E containing dexamethasone (1 μM), insulin (0.1 μM), and 10% FBS. The cells (1×10^6 cells) were placed in a 35 mm collagen type I coated culture dish (Iwaki, Funabashi, Japan) and incubated in humidified air with 5% CO_2 at 37 °C for 24 h.

Immediately preceding each experiment, the cell monolayer was washed with Hanks' medium containing 2.4 mM $CaCl_2$ and 10 mM HEPES (pH 7.4) and incubated in 2 ml of William's medium E containing 0.04% BSA (BSA/Williams) at 37 °C for 5 min. Phase contrast microscopy, utilizing an inverted system microscope (IMT-2, Olympus, Tokyo, Japan) was employed for observations of the cells. The cells were confirmed to be free of phagocytotic cells by their lack of Chinese ink uptake.

2.6. Preparation of liver nonparenchymal cells

Isolated liver cells were obtained from rats by collagenase perfusion as described above. Cells were centrifuged at 50 g for 2 min and the supernatant was further centrifuged at 500 × *g* for 5 min. The pellets were gathered and suspended in RPMI1640 containing 10% FBS. The cell suspension was seeded on non-coated 35 mm tissue culture plates. The cells were allowed to attach for 1 h at 37 °C in humidified air with 5% CO_2. Adherent cells were washed twice and incubated in humidified air with 5% CO_2 at 37 °C for 24 h. The cells were washed and used for the following experiments as liver nonparenchymal cells. After incubation with Chinese ink, microscopic observations indicated uptake of ink into the cells.

2.7. Binding experiments

Binding of polysaccharides to rat liver parenchymal and nonparenchymal cells was examined using ^{125}I-labeled polysaccharides as ligands. Incubation conditions allowing binding, but not uptake, were employed (Weigel and Oka, 1981), and binding strength was determined as previously reported (Tanaka et al., 1998). One milliliter of medium, containing ^{125}I-labeled ligand, was added to the washed monolayer of cells and incubated at 0 °C for 120 min. The media was aspirated

and the cells washed three times with ice-cold Hanks' medium containing 1.3 mM $CaCl_2$ and 10 mM HEPES (pH 7.4).

2.8. Uptake experiments

Polysaccharide uptake was determined using ^{125}I-labeled ligands incubated with rat liver parenchymal and nonparenchymal cells at 37 °C. To initiate uptake, 1 ml of pre-warmed (37 °C) BSA/Williams media, containing 1 μg/ml of ^{125}I-labeled ligand, was added to a pre-washed monolayer of cells, and incubated at 37 °C. At the appropriate time, the dishes were washed three times with ice-cold Hanks' medium containing 1.3 mM $CaCl_2$ and 10 mM HEPES (pH 7.4).

FITC-labeled polysaccharides (10 μg/ml) were added to the cell monolayer and incubated for 4 h. The cells were washed four times with ice-cold Hanks' medium containing 1.3 mM $CaCl_2$ and 10 mM HEPES (pH 7.4). The monolayers were examined by fluorescence microscopy (inverted microscope system, with IMT-2 epi-fluorescence apparatus attached, IMT2-RFC, Olympus, Tokyo, Japan). Photomicrography was carried out by an attached 35 mm single-lens reflex camera (OM-2, Olympus).

2.9. Sample analysis

Radioactivity and protein content were measured as previously described (Tanaka et al., 1998). One molar NaOH (1 ml) was added to the dishes, which were allowed to stand at room temperature for 20 min. A solubilized aliquot (0.7 ml) was neutralized with HCl and the radioactivity determined using a gamma counter, Aloka Auto Well Gamma System ARC-380CL (Aloka, Tokyo, Japan). The protein content was determined by the Lowry method (Lowry et al., 1951), using BSA as a standard. Data were obtained from three separate experiments with different cell cultures.

3. Results and discussion

Polysaccharides are one of the most attractive macromolecules for drug delivery. Their many hydroxyl groups provide for convenient drug attachment, and they display a unique distribution pattern in the body due to the sugar moieties. After intravenous injection, the dispositions of arabinogalactan, pullulan, dextran, and mannan, show distribution to, and accumulation within, the liver. The liver consists of approximately 80% parenchymal cells, and 20% nonparenchymal cells (Kupffer cells, endothelial cells, hepatic satellite cells and Pit cells). To elucidate the intracellular disposition of the polysaccharides, we examined the binding and uptake to the liver parenchymal and nonparenchymal cells, by the use of both radioisotope and fluorescence labeled polysaccharides.

3.1. Intracellular disposition in liver parenchymal cells

Fig. 1A shows the binding of ^{125}I-labeled polysaccharides, arabinogalactan, pullulan, dextran, and mannan, to liver parenchymal cells at 0 °C for 120 min, with arabinogalactan showing the strongest binding. The binding of arabinogalactan and pullulan significantly decreased upon addition of unlabeled polysaccharide, while dextran and mannan showed no effect. These observations indicate that arabinogalactan and pullulan are specifically bound to liver parenchymal cells, whereas dextran and mannan are not. A specific mechanism which has a saturable manner would contribute to the binding of arabinogalactan and pullulan.

To examine polysaccharide uptake, each of the ^{125}I-labeled polysaccharides was incubated with liver parenchymal cells at 37 °C for 120 min (Fig. 1B). Uptake of arabinogalactan and pullulan significantly decreased upon addition of unlabeled polysaccharide, whereas dextran and mannan were unaffected, indicating that arabinogalactan and pullulan uptake is specific and dextran and mannan is non-specific. Uptake of arabinogalactan and pullulan, as well as binding of them, would specifically occur by a saturable mechanism in liver parenchymal cells. It was also noted that polysaccharide uptake was larger than the respective binding. This suggests that the mechanism of uptake is temperature sensitive.

As arabinogalactan and pullulan displayed specific binding and uptake, we wished to further examine the mechanism for receptor-mediated endocytosis (RME). Arabinogalactan is bound and internalized to hepatocytes in vitro (Tanaka et al., 2004) and is known to distribute to the liver via the asialoglycoprotein receptor (Groman et al., 1994; Kaneo et al., 2000b). The asialoglycoprotein receptor may also contribute to

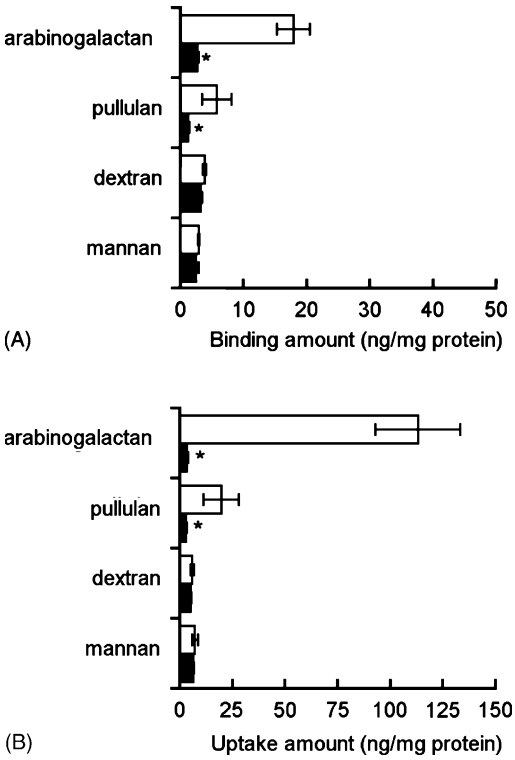

Fig. 1. Binding (A) and uptake (B) of arabinogalactan, pullulan, dextran, and mannan to liver parenchymal cells. Each ^{125}I-labeled polysaccharide (1 μg/ml) was incubated with the cells for 120 min. Incubation temperature of binding and uptake experiment were 0 and 37 °C, respectively. Open columns and closed columns represent the control and the co-incubation of unlabeled ligand (1000 μg/ml), respectively. Each column and horizontal bar represents the mean and standard deviation, respectively. *: There are static differences comparing with the value of each control with $p < 0.05$.

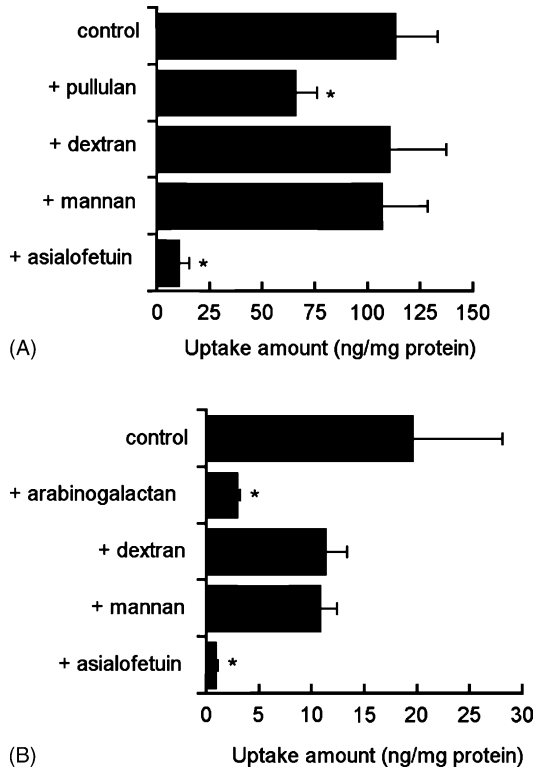

Fig. 2. Inhibition of uptake of arabinogalactan (A) and pullulan (B) to liver parenchymal cells by another polysaccharide or asialofetuin. Each ^{125}I-labeled polysaccharide (1 μg/ml) was incubated with the cells at 37 °C for 120 min as a control. Another polysaccharide or asialofetuin at the concentration of 1000 μg/ml was co-incubated. Each column and horizontal bar represents the mean and standard deviation, respectively. *: There are static differences comparing with the value of each control with $p < 0.05$.

pullulan distribution to the liver (Kaneo et al., 2001). Fig. 2 shows that arabinogalactan uptake to parenchymal cells significantly decreased on addition of pullulan or asialofetuin, just as uptake of pullulan decreases upon addition of arabinogalactan and asialofetuin. These results suggest that arabinogalactan and pullulan are absorbed into liver parenchymal cells via the asialoglycoprotein receptor and hence could serve as drug delivery agents to liver parenchymal cells via the RME mechanism because asialofetuin, a asisloglycoprotein and a ligand for the asialoglycoprotein receptor, inhibits the uptake of arabinogalactan and pullullan.

It has previously been shown (Enriquez et al., 1995) that use of an arabinogalactan conjugate (arabinogalactan with adenine arabinoside 5′-monophosphate) versus unmodified arabinogalactan does not reduce binding to the asialoglycoprotein receptor, and that the conjugate maintained antiviral activity. As our tyramine–arabinogalactan conjugate demonstrated binding and internalization to liver parenchymal cells, we make the same assumption that chemical modification did not influence RME (in the conjugation range 1.0–2.1, w/w%).

A pullulan-diethylenetriaminepentaacetic acid conjugate has shown effectiveness as a carrier of human interferon-β and plasmid DNA to the liver (Hosseinkhani et al., 2002; Suginoshita et al., 2002). Intravenous injections of these conjugates succeeded in specifically enhancing induction of an antiviral enzyme and in localizing the DNA plasmid to the liver. Our re-

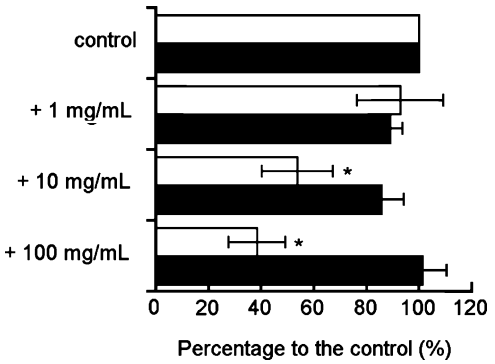

Fig. 3. Effect of excess amount of unlabeled dextran to the binding and the uptake of ^{125}I-dextran by liver parenchymal cells. ^{125}I-Labeled dextran (1 μg/ml) was incubated with the cells at 0 °C (open columns) and at 37 °C (closed columns) for 120 min. Each value represents the mean ± S.D. ($n = 3$). *: There are static differences comparing with the value of each control with $p < 0.05$.

sults would indicate that these liver distributions occur via the RME mechanism, as pulullan is distributed to liver parenchymal cells, through specific binding to the asialoglycoprotein receptor.

Dextran demonstrated binding and uptake to liver parenchymal cells. At concentrations of 1 mg/ml and 10 mg/ml, addition of unlabeled dextran had no effect on uptake (Figs. 1 and 3). At 1 mg/ml (Fig. 1) addition of unlabeled dextran had no effect on uptake, however, at 10 mg/ml (Fig. 3) binding was inhibited. Hence, binding of dextran to cells has an upper limiting concentration, whereas uptake is independent of concentration, implying that dextran adsorbs to the cell surface and is then taken up via fluid phase endocytosis which is independent of concentration. Nishikawa et al. (1992) investigated the disposition characteristics of dextran in mice and suggested the existence of a special uptake mechanism. Their pharmacokinetic results for the injection of dextran with galactosylated bovine serum albumin (Gal-BSA) suggested that dextran is taken up by the same mechanism as Gal-BSA. We examined the effect of arabinogalactan on binding and uptake of dextran in liver parenchymal cells (Tanaka, unpublished data) and found that dextran uptake is inhibited by the addition of arabinogalactan, suggesting that RME via the asialoglycoprotein receptor could contribute to the uptake of dextran by fluid phase endocytosis. A kinetic analysis of dextran uptake inhibition by asialofetuin would give more information about the role of receptor-mediated endocytosis, and is now in progress.

Dextran is a plasma expander and considered a passive, targeting drug carrier to tumors (Takakura and Hashida, 1995), where extended time spent in systemic circulation would result in increased delivery of drug to the tumor. Arabinogalactan inhibition of dextran uptake to liver parenchymal cells would help maintain a blood concentration of dextran.

3.2. Intracellular disposition in liver nonparenchymal cells

Fig. 4A shows polysaccharide binding to nonparenchymal cells. There was no suppression of bind-

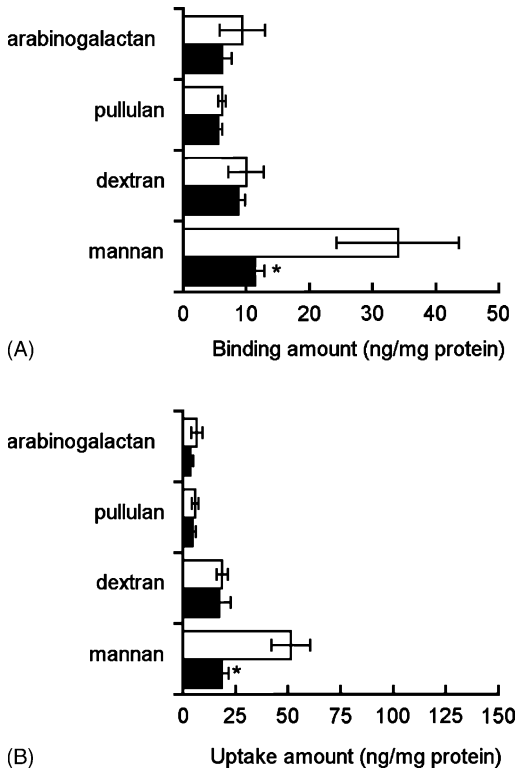

Fig. 4. Binding (A) and uptake (B) of arabinogalactan, pullulan, dextran, and mannan to liver nonparenchymal cells. Each ^{125}I-labeled polysaccharide (1 μg/ml) was incubated with the cells for 120 min. Incubation temperature of binding and uptake experiment were 0 and 37 °C, respectively. Open columns and closed columns represent the control and the co-incubation of unlabeled ligand (1000 μg/ml), respectively. Each column and horizontal bar represents the mean and standard deviation, respectively. *: There are static differences comparing with the value of each control with $p < 0.05$.

ing upon addition of excess amounts of unlabeled polysaccharide for arabinogalactan, pullulan and dextran. Arabinogalactan binding to nonparenchymal cells was approximately half that of the parenchymal cells, while pullulan binding to nonparenchymal cells was similar to that of parenchymal cells. Specific binding of arabinogalactan and pullulan was observed in the liver parenchymal cells, whereas non-specific was observed for nonparenchymal cells. Total and nonspecific binding of dextran to nonparenchymal cells was 2.6 and 2.7 times greater than to parenchymal cells, respectively. Total and nonspecific binding of mannan to nonparenchymal cells were 11.8 and 4.6 times greater than to parenchymal cells, respectively. Binding of ^{125}I-labeled mannan to liver nonparenchymal cells significantly decreased on addition of excess unlabeled mannan.

Fig. 4B shows polysaccharides uptake to nonparenchymal cells. There were no differences between the amount of binding and uptake for arabinogalactan and pullulan. However, dextran and mannan uptake was greater than binding. These suggest that an association of dextran and mannan to rat liver nonparenchymal cells would be depend on the temperature. The uptake of mannan to the nonparenchymal cells decreased on addition of excess unlabeled mannan. Binding and uptake to nonparenchymal cells was largest for mannan of the polysaccharides we investigated. Mannan also displayed stronger binding and uptake to nonparenchymal than to parenchymal cells, suggesting that a specific and saturable mechanism, mannose receptor-mediated endocytosis, would contribute to the association. The mannan receptor distributes in liver nonparenchymal cells, however there are few information about an intracellular disposition, e.g. in Kupffer cells and/or endothelial cells. We are now studying the intracellular disposition of mannan in reticuloendothelial cells.

3.3. Internalization of FITC-labeled polysaccharides to liver parenchymal and nonparenchymal cells

FITC-labeled polysaccharides were used to investigate the internalization of polysaccharides into liver cells. After incubation of parenchymal cells with FITC-labeled arabinogalactan or pullulan at 37 °C for 240 min, many fluorescent green granules were observed (Fig. 5A and B). Presence of the granules indicates that the hepatocytes internalized the FITC-labeled arabinogalactan. We also ascertained that FITC-labeled pullulan could be internalized into liver parenchymal cells in vitro. This observation, along with the binding and uptake results using ^{125}I-labeled pullulan, suggest that pullulan binds and internalizes into liver parenchymal cells via the asialoglycoprotein receptor.

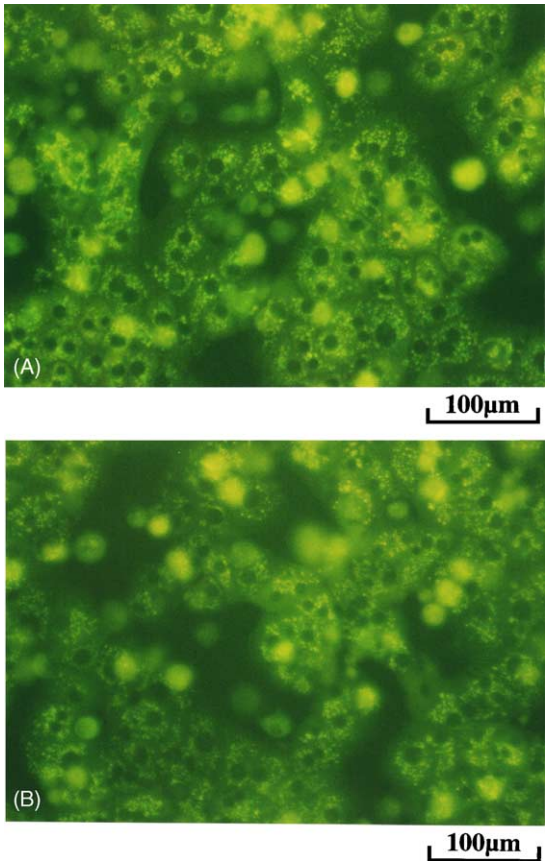

Fig. 5. Fluorescence microphotographs of liver parenchymal cells after incubation with FITC-labeled arabinogalactan (A) and pullulan (B). The cells were incubated with FITC-labeled polysaccharide at a concentration of 10 μg/ml. After 240-min incubation at 37 °C, the cells were washed with ice-cold Hanks' medium containing 1.3 mM $CaCl_2$ and 10 mM HEPES (pH 7.4) and were used for the microscope analysis.

FITC-labeled mannan interacted with liver nonparenchymal cells as shown in Fig. 6. This result, along with the binding and uptake experiments, suggest that a specific interaction between the liver nonparenchymal cells and mannan exist, probably involving the mannose receptor.

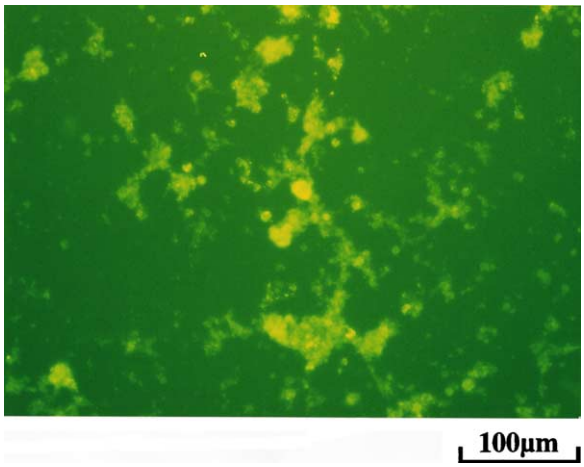

Fig. 6. Fluorescence microphotographs of liver nonparenchymal cells after incubation with FITC-labeled mannan. The cells were incubated with FITC-labeled mannan at a concentration of 10 μg/ml. After 240-min incubation at 37 °C, the cells were washed with ice-cold Hanks' medium containing 1.3 mM $CaCl_2$ and 10 mM HEPES (pH 7.4) and were used for the microscope analysis.

3.4. Relationship between the properties of polysaccharides and their disposition

Physicochemical properties, such as molecular weight and electric charge, as well as chemical conformation can affect the disposition of polysaccharides; although there are few reports on the relationships between them. Prescott et al. (1995) demonstrated that an arabinogalactan fragment (with a three- to four-fold lower molecular weight over the intact polysaccharide) showed no loss in binding potency to the asialoglycoprotein receptor. The hepatocyte adsorption of a cationic dextran–mitomycin C conjugate increased as the molecular weight of the dextran chain increased, while interactions between the anionic charged conjugate were negligible (Nakane et al., 1988). We reported that the hepatic uptake clearance in rats of FITC-labeled dextran decreased with increasing molecular weight (Kaneo et al., 1997). The effects of molecular weight on disposition are unclear and difficult to compare due to varying experimental conditions, e.g. in vitro or in vivo; different chemical modifications; varying analytical techniques. Chemical modifications to attach labels are usually required, as accurate, quantitative analysis of small quantities of naked polysaccharide is difficult. In this study, we used four polysaccharides which were labeled with a small amount of tyramine (less than 2.1, w/w%). The modification would not significantly affect the molecular weight or electric charge of the parent molecules, hence their effects can be discounted, as in the other studies described above.

In summary, sugars such as galactose, glucose, and mannose, in polysaccharides are highly effective for directing intracellular disposition. Furthermore, stereochemical configuration resulting from branching and hydroxyl group position, are key factors in intracellular disposition, as demonstrated with the differences between pullulan and dextran.

4. Conclusion

Our cell culture experiments demonstrate a pronounced difference in the intracellular disposition of various polysaccharides, with respect to binding and internalization to liver parenchymal and nonparenchymal cells. Arabinogalactan and pullulan were specifically taken up to liver parenchymal cells via the asialoglycoprotein receptor. Dextran was internalized to the cells by fluid phase endocytosis and the asialoglycoprotein receptor affected dextran binding to parenchymal cells. Conversely, mannan was specifically taken up to liver nonparenchymal cells. Clearly, stereochemical configuration, branching, hydroxyl group position, the various sugar residues and their arrangement within the polysaccharide structure all have a significant effect on their disposition properties. Defining the effects of compound structure on intracellular disposition is important in order to achieve specific drug targeting to liver cells, hence we are now studying the effects of drug conjugation with low- and macromolecular weight compounds on bio- and intracellular-disposition.

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