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Model Study of Hepatocyte Targeting Polymeric Super Molecular Assembly as Drug Carrier

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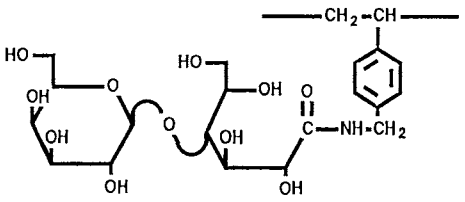
The drug binding properties of lactose carrying polystyrene (PVLA), which could be endocytosed into hepatocytes due to its interaction with receptors of asialoglycoprotein, were examined by UV and NMR spectroscopies. The results obtained with a hydrophilic drug model, 8-anilino-naphthalene-1-sulphonate (ANS), and with a hydrophobic drug model, 1,6-diphenyl-hexatriene (DPH), indicated the PVLA can be used as a hepatocyte targeting drug carrier.

Sugar moieties of glycolipid and glycoprotein on the cell surface are known to be important ligands which participate in recognition, cell-cell adhesion and information transfer between cells.[3,4] When sialic acids are removed from the oligosaccharide chain terminal of the native glycoproteins, the resulting asialo-glycoproteins are taken up from the blood circulation by hepatocytes and degraded by lysosome.[4] This clearance is mediated by specific receptors on the surface of the hepatocyte through the recognition of the galactose residues exposed by the removal of sialic acid.[4]

We found that PVLA, a synthetic model of asialoglycoprotein, promotes hepatocyte adhesion when coated on polystyrene culture dishes.[1,2] The hepatocyte adhesion was a galactose-specific threshold event effected by the density of the galactose immobilized on polystyrene as well as by the capping of the receptors.[5] This calcium dependent adhesion was inhibited by asialoglycoprotein, galactose and colchicine.[5]

These results indicated that the specific adhesion of hepatocytes is very similar to the interaction of asialoglycoprotein with its receptor indicating that the adhesion is mediated through the asialoglycoprotein receptors.[1,2,5] More interestingly, it was found that the hepatocytes adhering specifically on PVLA migrated together to form a high-density multilayer aggregation when growth factors were added to the culturing medium.[1]



Poly-N-p-vinylbenzyl-D-lactonamide:PVLA

Noticing the similarity between the interaction of asialoglycoproteins and PVLA with hepatocyte, we showed that the endocytosis of PVLA into rat hepatocytes was definitely possible with fluorescent labeled PVLA.[1,2,6] In addition, PVLA has the ability to bind hydrophobic and hydrophilic drugs because of its amphiphilic property due to the presence of both hydrophilic carbohydrate and hydrophobic vinylbenzyl moieties.[7] It had been reported that other polymer systems containing oligosaccharides have a strong affinity for organic solutes in water.[7,8] In this study, the binding of two drug models with PVLA was investigated to evaluate the possible application of PVLA in a target-specific drug delivery system.

N-p-Vinylbenzyl-[O-β-galactopyranosyl-(1-4)]-D-gluconamide (VLA) was prepared from p-vinylbenzyl chloride according to the published procedure.[7] Its polymerization was carried out in water at 60 °C with potassium peroxydisulfate ($K_2S_2O_8$) as the initiator. The polymer obtained was separated into fractions by ultrafiltration according to the molecular weight. PVLA with the molecular weight range of 30 kD to 50 kD was used for the binding study. The binding of the drug models were measured at 25 °C with a Shimazu UV2500 spectrophotometer, in phosphate buffer at pH 7.4. The concentration of PVLA, calculated from the molecular weight of the monomer, was fixed at 4.7 mM, while that of the drug models were changed between 0 mM and 5.6 mM. The difference spectra between model drug in water and in aqueous PVLA solution for DPH and ANS were shown in Fig. 1a and 1b, respectively. The interaction between PVLA and drugs occurred immediately after mixing. When 30 μl of a solution of DPH in ethanol was added to the PVLA solution (3 ml), DPH showed a spectrum which was not observable in aqueous solution without PVLA. This result suggested that PVLA can solubilize hydrophobic drugs in water. The interaction between PVLA and ANS was previously investigated by spectrofluorimetry, Kobayashi *et al.*[7] PVLA induced a blue-shift of the main 525 nm ANS fluorescence by 45 nm. This shift suggested that ANS was bound to a slightly hydrophobic region of the polymer. Figure 1b also showed the appearance of red shifted peaks at 287 nm and 391 nm showing the interaction between PVLA and ANS by UV spectrophotometry, and the absorbance

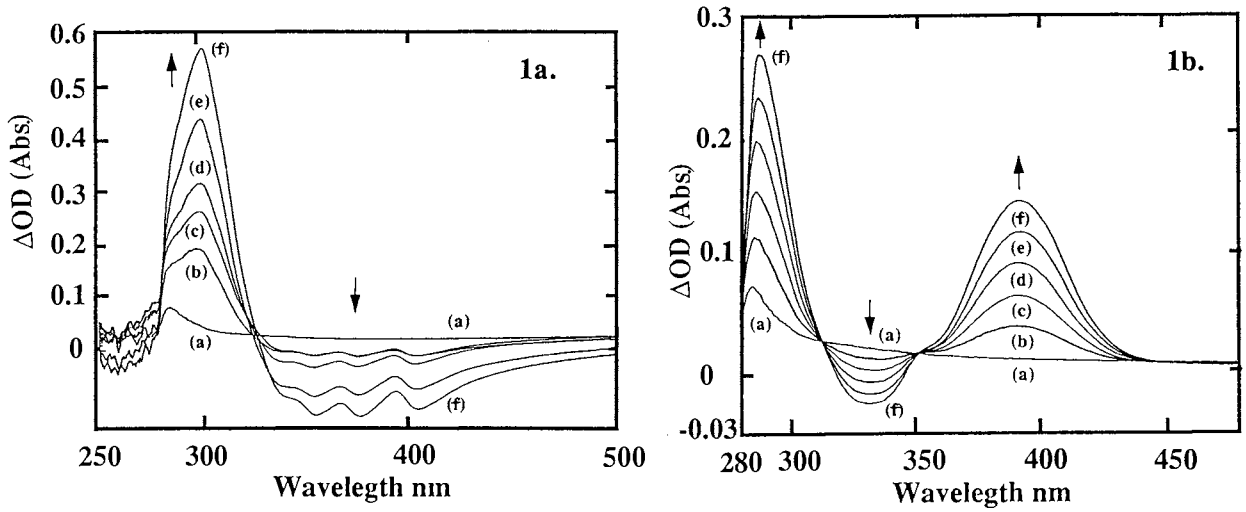


Fig.1a. Difference absorption spectra of DPH in aqueous PVLA solution and in aqueous solution. PVLA concentration was fixed at 4.7×10^{-3} mol/l, in the sample compartment. DPH concentrations were (a) 0; (b) 1.1×10^{-5}; (c) 2.3×10^{-5}; (d) 3.4×10^{-5}; (e) 4.5×10^{-5}; (f) 5.6×10^{-5} mol/l at 25 °C

1b. Difference absorption spectra of ANS in aqueous PVLA solution and in aqueous solution. PVLA concentration was fixed at 4.7×10^{-3} mol/l, in the sample compartment. ANS concentrations were (a) 0; (b) 1.1×10^{-5}; (c) 2.3×10^{-5}; (d) 3.4×10^{-5}; (e) 4.5×10^{-5}; (f) 5.6×10^{-5} mol/l at 25 °C

shift of ANS demonstrated that the environment of ANS was changed in the presence of PVLA molecule.

The Z-values of the ANS in the several solvents with different polarity had been used for the quantitative estimation of the micelle, liposome, and protein binding site polarity.[9] In these studies, the changes in the absorption of ANS when incorporated into micelles, liposomes, and protein were used to estimate the polarity of the binding site by interpolation using the plot of change in absorption of ANS versus the Z-value.[9] The ultraviolet spectrum of ANS absorption was measured in various ethanol-H_2O mixture and the change in the absorption maximum was plotted against Z-value for each mixture to determine the polarity of ANS binding site on PVLA. The change in ANS absorption in PVLA was found to be similar to the change of ANS in ethanol, suggesting that ANS is located in a binding site of PVLA which has same polarity as ethanol (Fig. 2).

The interaction between PVLA and ANS was also investigated by proton NMR spectrometry. The proton NMR spectrum of ANS dissolved in D_2O in side a capillary placed in a NMR tube containing PVLA solution in D_2O was compared with the spectrum of ANS in a solution of PVLA in D_2O (Fig. 3). The peaks of ANS (6.7-8.3 ppm) in the PVLA solution showed substantial shift and broadening indicating an interaction with PVLA. This interaction remained

even when the temperature was raised to 60 °C indicating that the complex formed between ANS and PVLA is very strong.

We investigated the interaction between PVLA and hepatocyte parenchymal cell *in vitro, ex vivo, and in vivo* by using fluorescent labeled PVLA.[10] PVLA was incorporated into hepatocytes by receptor mediated endocytosis. In this study, we observed the formation of super molecular assembly of PVLA and drug models which were bound to hydrophobic or hydrophilic regions of PVLA. These results clearly suggested that PVLA can be applicable as a useful drug carrier for hydrophobic and hydrophilic drugs targeted to hepatocytes.

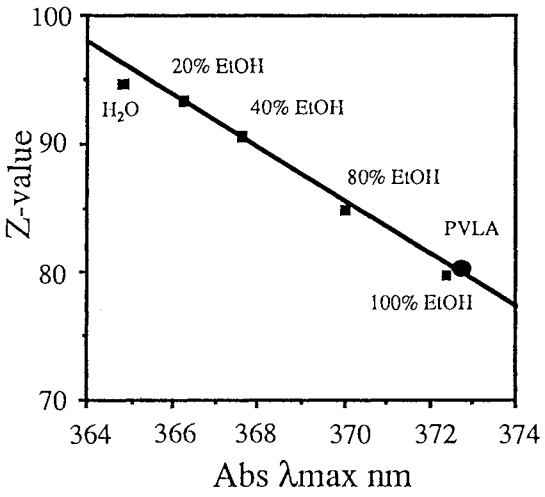


Fig.2 Change in the absorption of ANS vs. Z-value of several solvents. The Z-value of PVLA was estimated by plotting change of ANS (O) on this line.

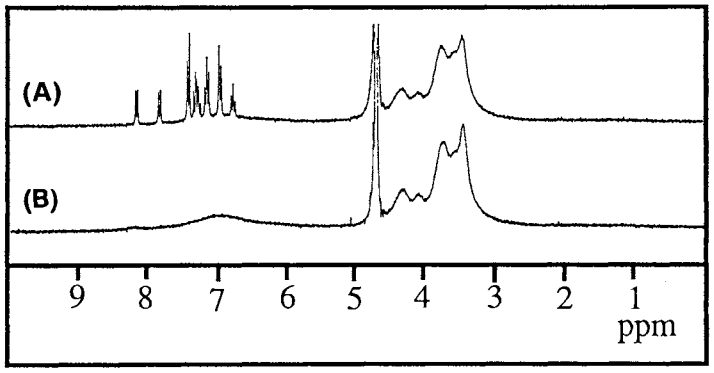


Fig.3 Proton NMR spectra showing intermolecular interaction between PVLA and ANS.
(A) 0.08 mg of ANS in D_2O in a capillary in a NMR tube with 1.54 mg of PVLA in 0.7 ml of D_2O .
(B) 0.08 mg of ANS in 1.54 mg/0.7 ml PVLA solution in D_2O.

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