Effect of ligand orientation on hepatocyte attachment onto the poly(N-p-vinyl benzyl-o-β-D-galactopyranosyl-D-gluconamide)

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ABSTRACT

The orientation effect of galactose ligand on hepatocyte attachment was investigated. Poly(N-p-vinyl benzyl-o-β-D-galactopyranosyl-D-gluconamide) (PVLA), a β-galactose-carrying styrene homo-polymer , was used as a model ligand for the asialoglycoprotein receptors on hepatocytes. PVLA was transferred onto the poly(γ-benzyl L-glutamate)(PBLG) or PBLG/ poly(ethylene glycol)(PEG)/PBLG Langmuir-Blodgett (LB) films as the monolayer level. The dichroic fluorescence values of confocal microscope indicated that the PVLA transferred onto the LB films was located with a preferential orientation of its molecular axes with regard to the direction of the α-helix of polypeptide. Hepatocyte recognized well-oriented galactose moieties of the surface of PVLA through asialoglycoprotein receptors.

INTRODUCTION

Extracellular matrices such as collagen, laminin, proteoglycan and fibronectin have been found to control spreading, migration, adhesion, and proliferation of cells since they have several globular domains which are specialized for binding to a particular molecule or cell[1]. Recently, many researchers have attempted to find out artificial cellular matrix instead of natural extracellular matrices whose structures and functions are rather complicated[2]. Akaike et al. already reported that poly(N-p-vinyl benzyl-4-o-β-D-galactopyranosyl-D-gluconamide)(PVLA) is one of the artificial celluar matrix for hepatocyte culture. They also reported that the adhesion is mediated by the galactose-specific interactiions between hepatocyte and PVLA which carries β-galactose ligands along the polymer chain[3].

The specific interactions between cells and ligands on the polymer surface are greatly affected by the ligand properties, such as ligand density[4], ligand distribution[5] and ligand orientation.

In this study, we are interested in the effect of ligand orientation on hepatocyte attachment onto the PVLA which was transferred onto the poly(γ-benzyl L-glutamate)(PBLG) or PBLG/poly(ethylene glycol) (PEG)/PBLG(GEG) block copolymer Langmuir-Blodgett(LB) films, which was little reported about the ligand orientation on the cellular behavior.

In a previous studies[6,7], it was found that the surface microstructure of block copolymers fabricated through LB method influenced cellular interactions. LB surface with a layered structure adhered a great number of cells than cast surfaces with microphase-separated domains. Also, extensive morphological changes of the platelets adhered onto the LB surfaces were observed when compared to the cast films.

MATERIALS AND METHODS

Materials

PVLA was synthesized as described by Kobayashi et .al.[8]. PBLG was sythesized from γ-benzyl L-glutamate N-carboxyanhydride(γ-BLG NCA), using triethylamine as an initiator in 1,4-dioxane. PBLG/PEG/PBLG(abbreviated as GEG) were

synthesized by initiating the polymerization of γ-BLG NCA with diamino terminal PEG, as the previously similar method described[9]. Bovine serum albumin(BSA) and fluorescein isothiocyanate(FITC) were purchased from Sigma Chemical Co. and used without purification. Anti-human asialoglycoprotein receptor was kindly supplied dy Daiichi Chemical Pharmaceutical Co. PVLA labeled with FITC was prepared by reacting PVLA(1g) with FITC(50mg), dibutyltin dilaurate(15mg), and one drop of pyridine in dimethyl sulfoxide(5ml) at 60°C for 12h, followed by precipitation onto ethanol and dialysis through a cellulose tube against distilled water. About one FITC molecule was introduced to 100 structural units in PVLA.

Preparations of LB films

To form LB films, PBLG homopolymer or GEG block copolymers were dissolved in chloroform(0.01% w/v) and spread onto the surface of double-distilled water to form monolayers. Surface pressure measurements were carried out at 21±1°C using a procedure described previously[10]. The monolayers were transferred onto silane-treated glass-plates by a horizontal lifting method at various surface pressures. One layer was transferred as X-type in this experiment.

To form PVLA films, the PVLA aqueous solution(1×10^{-3}M) was added in the subphase instead of pure water. Then, the PVLA film complexed with PBLG(or GEG) at air/water interface was transferred onto silane-treated glass plates. One layer was transferred.

Confocal laser microscopic observation of FITC-PVLA film

Fluorescence topography of FITC-PVLA transferred to PBLG LB film were obtained with polarized light parallel and perpendicular to the compression direction using a confocal laser microscope(ACAS 570, Meridian Co., USA).

Hepatocyte adhesion

PBLG(or GEG) and PBLG/PVLA(or GEG/PVLA) film prepared by the LB technique were immersed in hepatocytes which were isolated from the liver of female Sprague Dawley rats(150-200g) using the two-step collagenase perfusion technique of Seglen. The cell density was adjusted to $1X10^5$cells/ml in William's medium E without serum and the polymer films were placed in an incubator with 5wt% CO_2 incubator. After a prescribed time, the medium including free, non-adhered cells were throughly washed with a PBS solution. The number of collected free cells was counted by Coulter counter. The experiments were repeated at least three times to obtain reliable results.

RESULTS AND DISCUSSION

From the fluorescence spectroscopy, PVLA was adsorbed onto the PBLG(or GEG) LB film as the monolayer level. Also, it was found that the adsorbed PVLA increased with increasing surface pressure for the same PBLG because the hydrophobic interaction between PVLA and PBLG increased with increasing surface pressure. It can be said that polymer-polymer complex between PVLA and PBLG(or GEG) was formed at air/water interface.

From the topography of the confocal laser microscope of FITC-PVLA transferred onto the PBLG LB layer at 15 dyne/cm surface pressure with polarized light parallel and perpendicular to the compression direction, the fluorescence values of the PVLA for light polarized parallel are much larger than the values for light polarized perpendicularly to the compression direction. The dichroic ratio (estimated from the fluorescence color values) $V_{\parallel}/V_{\perp}$ was 15.6 for the PVLA transferred onto PBLG LB film at 15dyne/cm. This indicates that the PVLA is oriented preferentially with the longest axis normal to the PBLG α-helical axis. But it is not clear that the oriented PVLA in the PBLG α-helical axis adopts helical structure.

From hepatocyte adhesion onto PBLG(or GEG) and GEG/PVLA LB films in the absence of serum, it was found that not much differences of hepatocyte adhesion between PBLG(or GEG) and PVLA LB surfaces were found due to the non-specific interaction between cells and hydrophobic LB surface. But it was found that a little more hepatocytes were adhered onto the PVLA LB surface than PBLG(or GEG) one in any system.

From effect of albumin pretreatment(conc. of albumin:

0.04 wt-% and time: 1h) of the LB surface on hepatocyte adhesion onto PBLG(or GEG) and PVLA LB films in the absence of serum after 15 min, hepatocyte adhesion for the PBLG and GEG LB surfaces decreased after albumin treatment whereas its adhesion for the PVLA LB one was not changed after albumin treatment. It can be explained that albumin treatment of PBLG and GEG LB surfaces can block the non-specific interaction between cells and hydrophobic surfaces. Also, it can be said that hepatocytes recognize well-oriented galactose moieties on the surface of PVLA transferred onto the PBLG(or GEG) polymer LB films. It was found that about 25.3 ng/cm^2 of PVLA was adsorbed onto the PBLG LB film at the 5 dyne/cm of surface pressure, indicating monolayer level. But only 32.5 wt.-% of hepatocyte was adhered onto the PVLA-coated polystyrene dishes as control, which had 100 times of galactose density in comparison with LB film. Much more hepatocytes were adhered onto the PVLA LB surfaces, which had very low density of galactose moieties as compared to coating surfaces. Those differences of adhesion can be attributed to the well-oriented galactose moieties of PVLA LB surface, which is much easier for cells to recognize surface of asialoglycoprotein. As a matter of fact, the dichroic fluorescence values of confocal microscope indicated that the PVLA was located with a preferential orientation of its molecular axes with regard to the direction of the α-helix of polypeptide.

From effect of antibody on hepatocyte adhesion onto PBLG(or GEG) and PVLA LB films after 15 min, it was found that inhibition did not occur for the PBLG(or GEG) polymer LB surfaces whereas asialoglycoprotein antibody inhibited cell adhesion for the PVLA LB ones. Therefore, it can be said that adhesion of hepatocytes to the oriented PVLA LB surface is mediated through asialoglycoprotein receptors. The morphology and function of the adhered hepatocytes onto the PVLA LB surfaces will be reported in the near future.

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