論文内容の要旨

論文題目　Study of polymeric nanocarrier design on the membrane transporter mediated-targeting on the central nervous system

(中枢神経系を標的としたトランスポーター介在薬剤送達システムの基礎的解析)

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Active targeting mediated by ligand molecules has been attracting great attention in the field of drug delivery system (DDS) for the purpose of improving the selectivity to diseased tissues and cells [1]. Small compounds directed to cell surface receptors and antibodies binding to proteins overexpressed on targeted cells are principally considered as ligand molecules for promoting targeting. Recently, emerging ligand strategies targeting membrane transporters by using the small molecules that pass through them have attracted much attention. Small molecules which have low binding affinity to the targeted transporters can be utilized as a ligand by taking advantage of multivalent recognition on the surface of nanoparticulated DDS carrier [2]. Therefore, it is crucial to have a versatile platform that allows effective ligand presentation on the surface for achieving multivalent binding of ligands. Polymeric micelles are one of the most comprehensive platforms that can allow precisely controlling ligand installation, as well as being used for DDS to several diseases, such as cancer and central nervous system (CNS) disorders. Previously, glucose-installed polymeric micelles achieved the targeting of cancer cells and brain tissues crossing the blood-brain barrier (BBB) by recognizing glucose transporter-1 (GLUT-1) [3]. However, there still remains uncovered structural parameters of ligand installed polymeric micelle which affect to the function of polymeric micelle as a biomaterial and the target recognition ability of ligand molecules. Here, a versatile micelle platform was focused, *i.e.*, polyion complex micelles (PIC/m), which permits engineering various parameters affecting ligand installation and recognition. To apply PIC/m to this research, the formulation was first optimized to present prolonged blood circulation *in vivo*. This was achieved by fine-tuning the charge mixing ratio of

oppositely charged segments that compose the block copolymers forming the micelle. Then, the effect of chain length of the hydrophilic segment in the block copolymers and the influence of the spacer between the ligand and the shell of the micelles on the target recognition were investigated to develop formulations with enhanced delivery to brain tissues.

First, the structural characteristics of PIC/m prepared with varying charge mixing ratio in oppositely charged segment in the core was described. Although it is widely known that numerous parameters, such as the ionic strength of solvents [4, 5] and environmental pH [6], as well as the degree of polymerization (DP) of block copolymers [7], affect the physical properties of PIC/m as a versatile biomaterial, the effect of charge mixing ratio of core forming oppositely charged segment has not been investigated yet. In this research, PIC/m was prepared with anionic charged block copolymer, PEG-poly(α, β-aspartic acid) (PEG-PAsp, Mw of PEG = 2,200 Da, DP of PAsp = 80), and cationic charged block copolymer, PEG-poly([5-aminopentyl]-α, β-aspartamide) (PEG-P(Asp-AP), Mw of PEG = 2,200 Da, DP of P(Asp-AP) = 76), and then narrow distributed PIC/m was obtained with diameters ranging from 30 nm to 50 nm at [carboxyl]/[amine] (C/A) from 0.85 to 1.15. Hydrophilic surface properties, such as size distribution, morphology and surface potential, and the structural properties attributed by the core-forming segment, were investigated. Note that PIC/m prepared with the different radius of gyration measured by the small angle X-ray scattering and mobility of core segment molecules evaluated by proton nuclear magnetic resonance depending on the charge mixing ratio. Moreover, the performance of PIC/m with different structural features in the core in the biological system were evaluated, and then the essential parameters of PIC/m as a DDS carrier were proposed. The optimal charge mixing ratio of PIC/m, C/A = 1.05, that inhibits the adsorption to the liver sinusoidal walls, and prolongs the circulation in the bloodstream was identified [8].

Next, based on the optimized PIC/m preparation condition**,** the target efficiency of glucose-installed PIC/m (G-PM) as a DDS carrier was evaluated, particularly focusing on their ability to actively penetrate BBB by targeting GLUT-1 *via* glucose ligand molecules. For the purpose of developing the versatile platform of DDS effectively penetrating BBB, it is necessary to obtain quantitative structural parameters that affect the multivalent target recognition of G-PM. The crucial parameters that determine the binding affinity of G-PM to GLUT-1 were defined, as follows: 1) chain length of hydrophilic segment, which is poly (ethylene glycol) (PEG) in this research, and 2) distance between two ligand molecules on the surface of micelles. In this research, G-PMs with hydrophilic segment of PEG at a molecular weight of 2-, 5- and 12-kDa, and then target recognition of glucose ligand molecules on the surface of G-PMs was evaluated both *in vitro* and *in vivo*. From the surface plasmon resonance measurement, it was confirmed that ligand number on the surface of G-PM determined the binding affinity of G-PM to glucose binding lectins concanavalin A. As well as ligand density, length of PEG segment was clarified to be a

crucial parameter which controlled the G-PM recognition of GLUT-1 expressing on the surface of cancer cells and rat primary brain capillary endothelial cells *in vitro*. Long PEG chains with a molecular weight of 12 kDa decreases the target recognition of ligand molecules due to high steric repulsion compared to short PEG chains with a molecular weight of 5- and 2-kDa. G-PM with 2 kDa PEG achieved dramatically increased target recognition when a distance between two ligand molecules was less than 10 nm, so that G-PM with 2 kDa PEG obtains the multivalent effect with higher ligand density.

Finally, G-PM were further engineered with the technique called cocktail PEGylation, which allows increasing the target recognition of ligand molecules conjugated to longer PEG chain by mixing shorter PEG chain as a spacer [9]. G-PM with 12 kDa PEG could not achieve ligand recognition, but longer PEG chain is preferable for stable micelle formation so that ligand conjugated-block copolymers containing 12 kDa PEG and block copolymers containing 2 kDa PEG as spacer were chosen for the preparation of cocktail PEGylated G-PM (cG-PM). Cocktail PEGylation dramatically improved the binding affinity to targeted molecules, as well as brain delivery of G-PM bearing the ligand molecules attached to 12 kDa PEG blocks. Increasing of short PEG mixing ratio ranging from 50 to 70 % occurred the increasing of target recognition of ligand molecules in the surface plasmon resonance measurement utilizing concanavalin A as a model target protein and *in vitro* cellular uptake assay with GLUT-1 highly expressing cancer cells and rat primary brain capillary endothelial cells. Note that, on the other hand, cG-PM with 60 % of short PEG showed the highest brain accumulation rate comparing with cG-PM with 50- and 70-% of short PEG because cG-PM with 70 % of short PEG stuck in the brain endothelial cells and could not penetrate BBB efficiently due to the high binding affinity to GLUT-1. These findings indicate that the mixing ratio of short PEG chain critically determines the binding affinity of cocktail PEGylated G-PM to GLUT-1. Thus, precise control of the short PEG chain ratio is required to increase BBB penetration of G-PM with suppressed stacking to brain endothelial cells because of restrained dissociation from GLUT-1 [10].

As a conclusion of this research, by analyzing the effect of structural parameters of PIC/m on the self-assembly process, ligand presentation and targeting capability in biological systems, current knowledge was much improved on the preparation of PIC-based nanoparticulated materials, which could allow more efficient and effective carriers with tunable structural and functional characteristics for controlling *in vivo* action. Moreover, the observations of this work provide general information on structural parameters of G-PM to enhance the accumulation in brain, as well as rationalize the structural design of DDS based on G-PM as a carrier of various types of drugs, such as nucleic acids [11] and antibodies [12], for the treatment of CNS disorders. Furthermore, these findings may contribute to other fields of bioengineering by providing useful structural guidelines for biomaterials employing multivalent ligand recognition.

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