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Cell Specificity of Polysaccharide Derivatives on Liposomal Surface

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Various pullulan derivatives, which have both cholesterol and another monosaccharide terminal such as hexosamines and 1-amino-hexoses, were synthesized and employed for coating liposome. The lectin-induced aggregation and the phagocyte uptakes of such polysaccharide-coated liposomes were effectively controlled by changing only the terminal sugar residue of polysaccharide derivatives.

Saccharide determinants play an important role in biological recognition such as antigen-antibody interaction and cell-cell adhesion. Recently, the receptor specific sugar moieties have been revealed.[1] For example, liver parenchymal cell has a receptor which can specifically recognize galactose,[2] phagocytic cells have receptor to mannose, [3] and fibroblasts have mannose-6-phosphate specific receptor.[4] Since 1982 we have been developing a methodology to achieve the receptor-mediated targeting in liposomal drug delivery system (DDS).[5-7] This method involves coating of the outermost surface of liposome with a cell specific polysaccharide derivative. We have already reported that several liposomes coated by naturally occurring polysaccharide derivatives show excellent physicochemical stability and significant cell specificity.[7] Moreover, even partial modification of the terminal moieties of pullulan by conjugating sialic acid was enough for controlling cell-specificity of the liposome.[8] In order to obtain more information about the relationship between the cell-specificity and the chemical structure of terminal sugar moiety of the polysaccharides, we newly synthesized pullulan derivatives which carry galactosamine, 1-aminogalactose, mannosamine, 1-aminomannose, glucosamine and 1-aminoglucose in part.

Pullulan (MW 50000, Hayashibara) was selected as the starting polysaccharide because of its less cell specificity and the highest coating efficiency.[7] Substitution procedure of cholesterol group to pullulan has been described elsewhere.[7] Pulullan derivative(CHP-50) so obtained was bearing approximately 1.0 cholesterol moiety per hundred glucose units and coded as CHP-50-1.0. Carboxymethylated CHP-50(CM-CHP-50) was obtained by the reaction of CHP(1.5 g,9.3

equiv. mmol) with excess sodium chloroacetate(7.9 g,68 mmol) in an aqueous solution for 24 h at pH 11.0 and 25 °C. The product was purified by dialysis using Seamless Cellulose Tubing(VISKASE SALES Corp.). The carbonyl group introduced was identified by ^{13}C-NMR (δ,177 ppm with TMS) on a JEOL JNM-GX-400 and by IR (KBr disk,$\nu_{C=O}$, 1600 cm^{-1} and 1410 cm^{-1}). The degree of carboxymethylation was estimated by potentiometric titration to be around 40-60 per 100 glucose units. Additional saccharide units were introduced to CM-CHP-50 by condensation with the amino sugars. Hexosamines were commercially available (Nakarai Chemicals). 1-Aminohexoses were synthesized by the method described in the literature.[9] Three hundred mg (1.6 mmol) of an amino sugar were added to a mixture of 150 mg (0.93 equiv. mmol) of CM-CHP and 300 mg (1.6 mmol) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide(EDC) dissolved in 10 ml of distilled water. The resulting mixture was reacted for 48 h at pH 6.5 and 25 °C, dialyzed for 3 days against 5 liter of water , and lyophilized. Amido group was detected by IR ($\nu_{C=O}$, 1650 cm^{-1}). The substitution degree of amino sugar to CM-CHP-50 was determined by elemental analysis. Structures of polysaccharide derivatives are shown in Fig. 1. The abbreviation of galactosamine-43-CHP-50-1.0 (Gal(2)) means, for example, pullulan (MW 50000) substituted with 43 galactosamines and 1.0 cholesterol moieties per 100 glucose units. All other polysaccharide derivatives were coded by the same manner.

First, we investigated a specific lectin-induced aggregation of the liposome coated with these chemically modified pullulan by employing ConA (concanavarin A,α-D-mannose and α-D-glucose specific lectin) and APA (Abrus precatorius lectin, β-galactose specific lectin). Pullulan derivative-coated multilamellar liposomes (MLV) were prepared according to the method previously described.[7] An aqueous suspension of MLV was mixed with an aqueous polysaccharide solution at the ratio of [polysaccharide]/[phospholipid] = 0.2 ~ 0.5 by weight. Aggregation of the liposomes

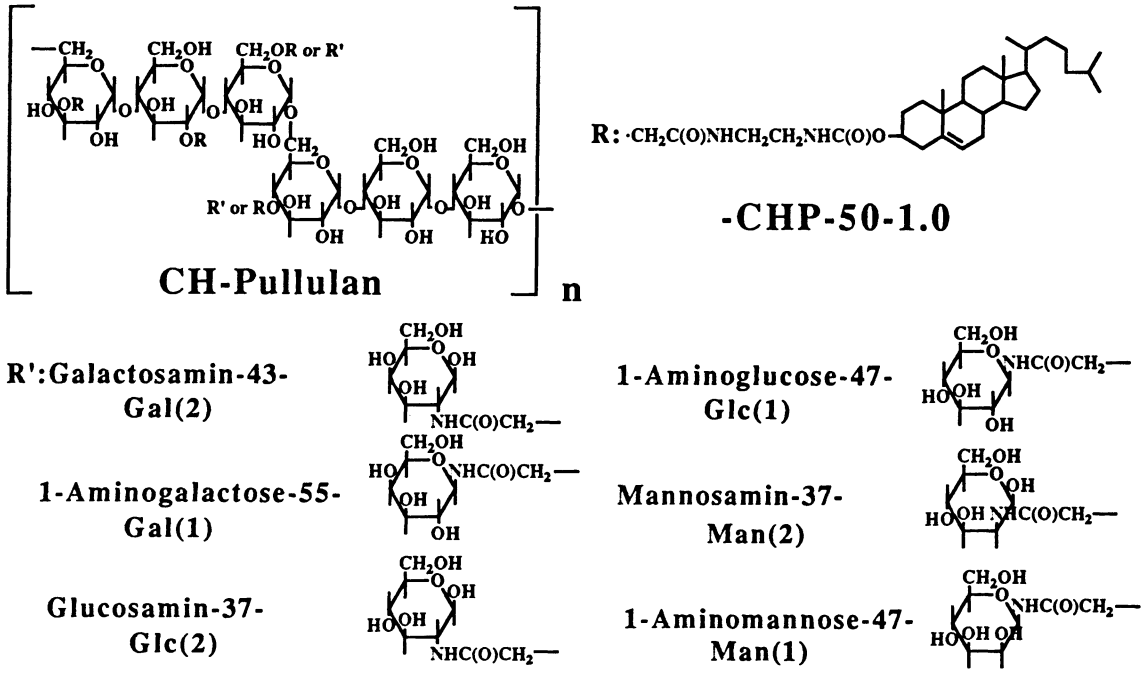

Fig.1. Structures of polysaccharide derivatives.

was followed by monitoring the turbidity increase(at 360 nm). Figure 2 shows ConA-induced aggregations of pullulan derivatives-coated liposomes. Extent of ConA-induced aggregation of the pullulan derivatives was the following sequence in terminal monosaccharides : 1-aminomannose(Man(1)) > 1-aminoglucose(Glc(1)) >> glucosamine(Glc(2)) > galactosamine(Gal(2)) > mannosamine(Man(2)) ≈ 1-aminogalactose (Gal(1)) ≈ 0. This sequence is coincident with that of specificity of ConA to monosaccharides reported.[10] On the other hand, in the case of APA-induced aggregation of them, the sequence in the specificity was rather different from that of the case of ConA-induced aggregation. The significant aggregation was observed for 1-aminogalactose-55-CHP-50-1.0(Gal(1))-coated liposome. As the result, the lectin-induced aggregation of the polysaccharide-coated liposomes was closely correlated with the specificity of lectin to the saccharide structure on the liposomal surface. This is a simple simulation of biological recognition phenomena such as cell-cell adhesion or intercellular transinformation by saccharide determinants.

Secondly, internalization efficiency of these polysaccharide-coated liposomes by phagocytes (human neutrophils and monocytes) was examined. Figure 3 shows the phagocyte uptake of these liposomes monitored by RI(radio isotope) method using [^{14}C]-DPPC(dipalmitoylphosphatidylcholine) labelled liposomes. Interestingly, both cell lines showed similar phagocytic activity for the various liposomes. The uptake was higher in liposomes coated by Gal(1), Gal(2), and Man(1) compared with conventional pullulan-coated liposome, while the cell uptake of Glc(1)-coated liposome was drastically low. It is noteworthy that the uptake of

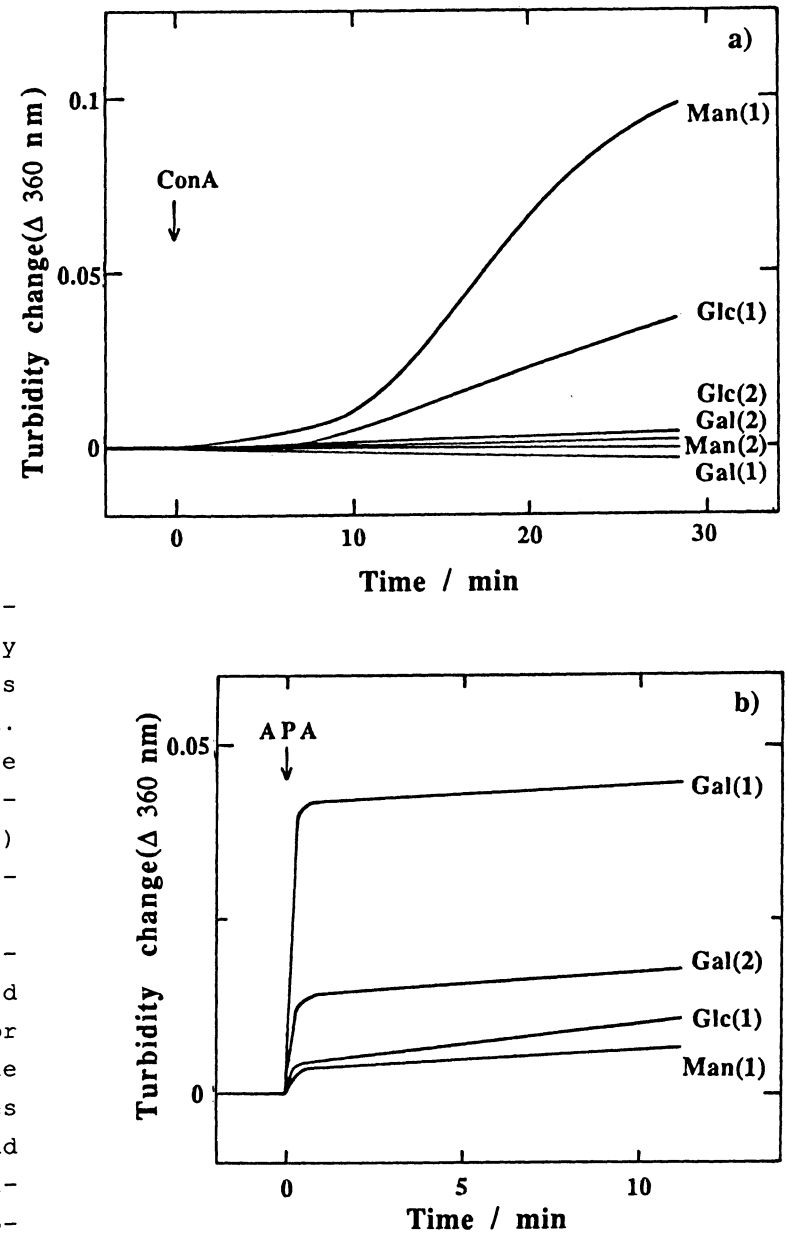

Fig.2. ConA-induced (a) and APA-induced (b) aggregation of various polysaccharide coated MLV at 37 °C in 20 mM Tris + 200 mM-NaCl (pH 7.4).

the polysaccharide-coated liposome by phagocytes was effectively controlled by changing only the terminal sugar residue of polysaccharide. Receptor mediated phagocytosis, especially regarding the cell recognizability of saccharide determinant, has been reported for various mammalian cells.[1] Results in this work may offer very useful information about membrane receptor of phagocytes. Moreover, the obtained information is very important not only in the development of cell specific biomaterials but also in the understanding of the role of saccharides in intercellular communications. The polysaccharide-coated liposomes seem to be useful probe for searching unknown receptor of various cells.

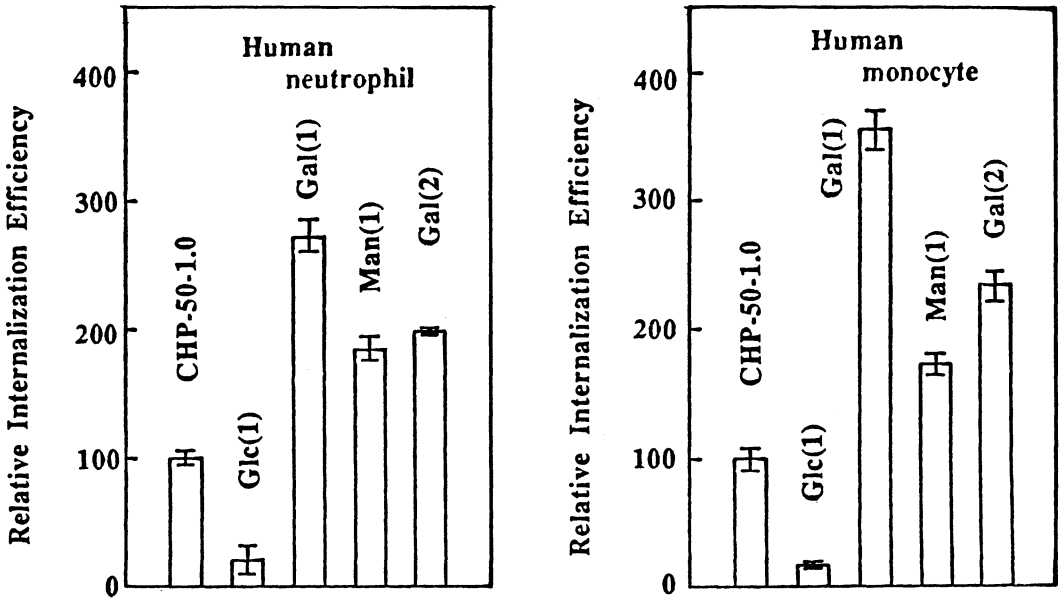

Fig.3. Relative internalization efficiency of various liposomes labelled with [^{14}C]-DPPC into human neutrophils and monocytes at 37 °C for 60 min in a culture medium(RPMI-1640, Nissui) containing 10% FBS(fetal bovine serum). [MLV]/[cell]=6.7x10^5.

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