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Bio-nanocapsules displaying various immunoglobulins as an active targeting-based drug delivery system

Kenji Tatematsu, Masumi Iijima, Nobuo Yoshimoto, Tadashi Nakai, Toshihide Okajima, Shun'ichi Kuroda *

The Institute of Scientific and Industrial Research, Osaka University, Ibaraki 567-0047, Japan

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ABSTRACT

The bio-nanocapsule (BNC) is an approximately 30-nm particle comprising the hepatitis B virus (HBV) envelope L protein and a lipid bilayer. The L protein harbors the HBV-derived infection machinery; therefore, BNC can encapsulate payloads such as drugs, nucleic acids, and proteins and deliver them into human hepatocytes specifically *in vitro* and *in vivo*. To diversify the possible functions of BNC, we generated ZZ-BNC by replacing the domain indispensable for the human hepatotrophic property of BNC (N-terminal region of L protein) with the tandem form of the IgG Fc-binding Z domain of *Staphylococcus aureus* protein A. Thus, the ZZ-BNC is an active targeting-based drug delivery system (DDS) nanocarrier that depends on the specificity of the IgGs displayed. However, the Z domain limits the animal species and subtypes of IgGs that can be displayed on ZZ-BNC. In this study, we introduced into BNC an Ig κ light chain-binding B1 domain of *Finegoldia magna* protein L (protein-L B1 domain) and an Ig Fc-binding C2 domain of *Streptococcus* species protein G (protein-G C2 domain) to produce LG-BNC. The LL-BNC was constructed in a similar way using a tandem form of the protein-L B1 domain. Both LG-BNC and LL-BNC could display rat IgGs, mouse IgG1, human IgG3, and human IgM, all of which not binding to ZZ-BNC, and accumulate in target cells in an antibody specificity-dependent manner. Thus, these BNCs could display a broad spectrum of Igs, significantly improving the prospects for BNCs as active targeting-based DDS nanocarriers.

Statement of Significance

We previously reported that ZZ-BNC, bio-nanocapsule deploying the IgG-binding Z domain of protein A, could display cell-specific antibody in an oriented immobilization manner, and act as an active targeting-based DDS nanocarrier. Since the Z domain can only bind to limited types of Igs, we generated BNCs deploying other Ig-binding domains: LL-BNC harboring the tandem form of Ig-binding domain of protein L, and LG-BNC harboring the Ig binding domains of protein L and protein G sequentially. Both BNCs could display a broader spectrum of Igs than does the ZZ-BNC. When these BNCs displayed anti-CD11c IgG or anti-EGFR IgG, both of which cannot bind to Z domain, they could bind to and then enter their respective target cells.

1. Introduction

Drug delivery system (DDS) nanocarriers control the spatiotemporal distribution of drugs in the body, enhancing their efficacy and reducing their side effects. In this regard, nanoparticles of approximately 100 nm in diameter have been widely used as DDS nanocarriers, because they can extravasate from leaky blood vessels at angiogenic or inflammatory sites in tumors [1]. Specifically, most conventional DDS nanocarriers are based on the enhanced permeability and retention (EPR) effect, and can therefore be described as passive targeting-based DDS nanocarriers.

While the EPR effect does not occur in all human tumors [2], anti-cancer drugs delivered using these passive targeting-based DDS nanocarriers are very effective in tumors where the effect does occur [3]. To deliver drugs even more precisely to specific tumors, or indeed to non-cancerous sites, the surface of these DDS nanocarriers can be modified using bio-recognition molecules, such as

* Corresponding author at: Department of Biomolecular Science and Reaction, The Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Osaka 567-0047, Japan.
E-mail address: skuroda@sanken.osaka-u.ac.jp (S. Kuroda).

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antibodies, ligands, peptides, and sugar chains, to establish active targeting [4].

The hepatitis B virus (HBV) specifically infects human hepatocytes, both *in vitro* and *in vivo*. The virus targets hepatocytes via a specific infection machinery that is harbored by the HBV envelope L protein, which has been synthesized as an approximately 30-nm nanoparticle (L particle) inside budding yeast cells [5]. Following electroporation in the presence of various payloads – drugs, genes, or proteins – these nanoparticles can deliver their contents to human hepatocytes *in vitro* at a rate comparable to that of HBV [6]. Furthermore, they can convey both genes and drugs to human hepatic tumors after intravenous injection in a mouse xenograft model [7]. Thus, the nanoparticles appear to act as active targeting-based DDS nanocarriers, and have come to be known as bio-nanocapsules (BNCs) [8]. Payloads have been incorporated into BNCs using chemical modification [9], liposomal fusion [10], and virosome formation [11]. With regard to the functional domains indispensable for the human hepatocyte-specificity of HBV, the N-terminal 108 amino acids (aa) of the L protein (pre-S1 region, serotype *ayw*) contains a sodium taurocholate cotransporting polypeptide (NTCP; a potential HBV receptor)-binding site (2–48 aa) used by the virus to attach onto human hepatocytes [12], and a fusogenic domain (9–24 aa) that allows the capsid of HBV to escape from the endosome [13]. The central region (109–163 aa; pre-S2 region) of the L protein contains a receptor for polymerized human serum albumin (120–129 aa); presumably, this prevents the opsonization of HBV [14,15]. The C-terminal 226 aa of the L protein (S region) contains 4 transmembrane domains for the self-assembly of the particle structure [16]. These functional domains likely contribute synergistically to the active targeting of both HBV and the BNC to the human liver, as well as ensuring escape from recognition by immune cells in the blood stream, cell entry by endocytosis, and endosomal escape [15].

The human hepatotrophic properties of the BNC have been altered by replacing the domains containing the NTCP-binding site with other biorecognition molecules such as ligands, peptides, and lectins [17]. For example, to diversify the active targeting ability of the BNC, we generated the ZZ-BNC by replacing most of the pre-S1 and pre-S2 regions (51–153 aa) with the tandem form of the IgG Fc-binding Z domain of *Staphylococcus aureus* protein A (protein-A Z domain) [18]. The ZZ-BNC tethers a maximum of 60 IgG molecules and displays the Fv region outwardly in an oriented-immobilization manner [19,20]. When anti-epidermal growth factor receptor (EGFR) antibodies are displayed on the ZZ-BNC, the nanoparticles accumulate in EGFR-overexpressing glioma cells after intraventricular injection [18]. Similarly, *in vivo*, ZZ-BNC displaying anti-CD11c antibodies accumulated in CD11c-expressing dendritic cells (DCs) after intravenous injection; this subsequently promoted DC maturation [21]. These results indicate that the ZZ-BNC can deliver payloads to specific sites both *in vitro* and *in vivo*, based on antibody specificity. The disadvantage of the ZZ-BNC is that the protein-A Z domain used can only bind to limited types of Igs. For example, the nanoparticle can only weakly bind to human IgG3, mouse IgG1, rat IgGs, and all IgMs [19]. These limitations of the ZZ-BNC have prompted us to generate BNCs possessing other Ig-binding domains; namely, the Ig κ light chain-binding B1 domain of *Finegoldia magna* protein L (protein-L B1 domain) [22,23], as well as the Ig Fc-binding C2 domain of *Streptococcus* species protein G (protein-G C2 domain) [24], both of which can display most Igs (IgG, IgM, IgE, IgA, and IgD). In this study, we have prepared two types of BNC: the LL-BNC, harboring the tandem form of protein-L B1 domain, and the LG-BNC, harboring the protein-L B1 domain and the protein-G C2 domain sequentially. We then characterized the biochemical and physicochemical properties of both types. Both display a broader spectrum of Igs than does the ZZ-BNC. When the BNCs display anti-CD11c IgG (Armenian hamster IgG, rat IgG2a), or anti-EGFR IgG (mouse IgG2a, mouse IgG1), they can bind to and then enter their respective target cells (*i.e.* a CD11c- or EGFR-positive cells, respectively).

2. Materials and methods

2.1. Preparation of the BNCs

To prepare a 0.2 kilo-base pair (kbp) DNA fragment encoding the protein-L B1 domain, the protein L gene of *F. magna* [25] was amplified using PCR, then the product was digested using either *Not*I or *Mlu*I. Similarly, the protein G gene of *Streptococcus* species was amplified using PCR [25], then the product was digested using *Mlu*I to produce a 0.2-kbp DNA fragment encoding the protein-G C2 domain. The plasmid pGLD-ZZ50, an expression plasmid for ZZ-L protein (Fig. 1) in yeast [18,26,27], was then digested using *Not*I to remove the DNA fragment encoding the protein-A Z domain. The 0.2-kbp *Not*I-digested DNA fragment encoding the protein-L B1 domain was then ligated into the plasmid to generate the pGLD-L plasmid. After digestion of the plasmid using *Mlu*I, the 0.2-kbp *Mlu*I-digested DNA fragments encoding either the protein-L B1 domain or the protein-G C2 domain were ligated into the plasmid to generate either the pGLD-LL or the pGLD-LG plasmid, respectively. These plasmids in turn express either the LL-L protein or LG-L protein (Fig. 1). Both the LL-BNC and LG-BNC were synthesized in yeast cells harboring either the pGLD-LL plasmid or the pGLD-LG plasmid, respectively. As previously reported [5], *Saccharomyces cerevisiae* AH22R$^-$ cells were transformed with each plasmid using the spheroplast method. Each transformant was cultured and subjected to BNC purification according to the published method [27,28]. Briefly, the yeast cells from the 4-L medium (wet weight: ~130 g) were disrupted using glass beads in the presence of 8 M urea, and then subjected to heat treatment (60 °C, 30 min). The supernatant was applied to an affinity chromatography column packed with human total IgG-immobilized Sepharose™ 4B (GE healthcare, Little Chalfont, UK), and subsequently to a gel filtration chromatography column packed with Sephacryl™ S-500 HR (GE healthcare).

2.2. Antibodies

Armenian hamster monoclonal anti-CD11c IgG (clone N418) was purchased from eBioscience (San Diego, CA). Rat anti-CD11c IgG2a (clone 223H7) and mouse IgG2a isotype control were purchased from Medical and Biological Laboratories (Nagoya, Japan). Mouse IgG2a anti-EGFR (clone 528 derived from ATCC HB-8509 strain) was purchased from Katayama Chemical Industries (Osaka, Japan). Mouse IgG1 anti-EGFR (clone LA1) and mouse IgG1 anti-EGFR (clone 225) were purchased from EMD Millipore (Darmstadt, Germany). Rat IgG2a anti-EGFR (clone ICR10) was purchased from Abcam (Cambridge, UK). Human IgG3, human total IgM, porcine total IgG, bovine total IgG, mouse IgG1 isotype control, rat total IgG, and rabbit total IgG were purchased from Sigma–Aldrich (Saint Louis, MO). Human total IgG was purchased from Equitech-Bio (Kerrville, TX).

2.3. Transmission electron microscopy (TEM)

The BNCs (2 μg/ml as L protein) were adsorbed onto a carbon-coated copper grid (JEOL, Tokyo, Japan), negatively stained using 2% (w/v) phosphotungstic acid (pH 7.0), and observed under TEM using a JEM™ 1011 (JEOL).