Abs. 2615, 206th Meeting, © 2004 The Electrochemical Society, Inc.

THE POTENTIAL OF PEPTIDE-CONJUGATED LIPOSOMES TO BE USED FOR OPTICAL SENSING OF SPECIFIC TARGETS

Takako Nishiya and Chikaho Toma
Research & Development Division
Fujirebio Inc.
51 Komiya-cho, Hachioji, Tokyo 192-0031, JAPAN

Fluorescence molecular tomography (FMT) is a recently developed imaging technology that can be used to localize and quantify fluorescent probes three-dimensionally in deep tissues at high sensitivities. Clinical FMT imaging applications will ultimately require biocompatible near-infrared probes and highly efficient photon collection systems, but penetration depths of 7 to 14 cm are theoretically achievable depending on the tissue type. Present study examined the feasibility of using peptide-conjugated liposomes for the detection of thrombus containing activated platelets in deep veins using the FMT imaging technique, and for the delivery of therapeutic substances to the thrombus.

For this purpose, liposomes with a covalently bound synthetic peptide containing the dodecapeptide sequence HHLGGAKQAGDV, the putative platelet interaction site at $\gamma^{400\text{-}411}$ of fibrinogen (dodecapeptide-liposomes), were prepared. These liposomes enhanced platelet aggregation and specifically adhered to platelets activated on the collagen surface. Dodecapeptide-liposomes released encapsulated materials upon interaction with platelets activated on the collagen surface. The rate of content release was dependent on the peptide surface density (Fig. 1). Liposome content release was not observed by the addition of anti-dodecapeptide antibody into a homogeneous solution of dodecapeptide-liposomes, indicating that the interaction between the dodecapeptide-liposomes and platelets activated on the collagen surface induces clustering of the surface-coupled ligands at the binding site on the receptor matrix to facilitate release of the internal contents through the liposome membranes. The level of lipid mixing between the dodecapeptide-liposomes and platelets activated on the collagen surface was relatively low, however it was increased in liposome preparation containing octa-arginine, the $(R)_8$GDV sequence, while content release was maintained at the same level as that of the dodecapeptide-liposomes (Figs. 2 & 3). The level of content release and lipid mixing for liposome preparations containing the RGD sequence as a ligand (RGD-liposomes) upon interaction with platelets activated on the collagen surface was extremely low. Both the level of the content release and lipid mixing, however, were enhanced in liposome preparations containing octa-arginine, the $(R)_8$RGD sequence (Figs 2 & 3). Dodecapeptide-liposomes and RGD-liposomes were not internalized by activated platelets. On the other hand, liposomes containing $(R)_8$PPQ, $(R)_8$RGD, or $(R)_8$GDV were internalized by activated platelets, and the extent of internalization was inversely related to ligand affinity to the target, suggesting that the use of the arginie chain enhances the delivery of the liposome content to the inside of the platelets. These results suggest that liposomes containing ligand with a lower affinity for the target, such as liposomes containing $(R)_8$PPQ, are internalized as intact liposomes, and therefore, there is efficient aqueous content mixing inside the platelets. For liposomes containing ligand with a higher affinity for the target, aqueous content mixing is less efficient due to the rapid extracellular release of the liposome content.

These results demonstrate the feasibility of using peptides to direct liposomes to molecular targets, regulating the mode of interaction with the target. Furthermore, indocyanine green (ICG), which is approved for human use by the US Food and Drug Administration, is practically inert to conditions for the incorporation into an aqueous phase of liposomes, and absorbs in the near-infrared region.

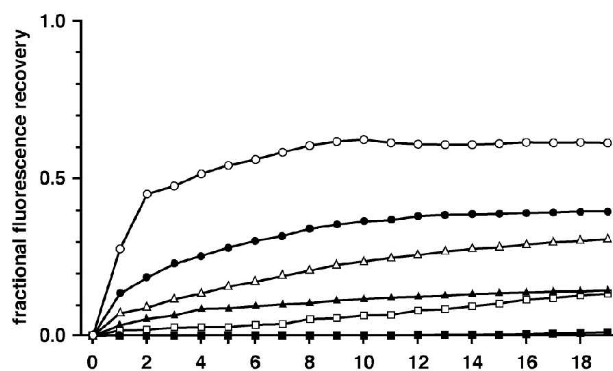

Fig. 1. Effect of surface density of dodecapeptide on calcein release from liposomes. Peptide surface density (molecules/particle); 27000 (open circle), 19000 (solid circle), 7500 (open triangle), 5900 (solid triangle), 4600 (open square), 0 (solid square).

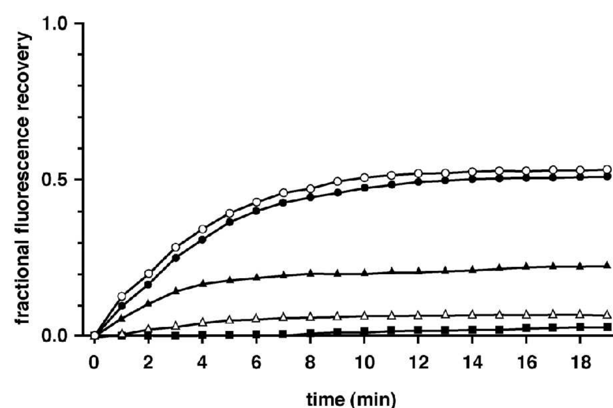

Fig. 2. Calcein release from liposomes depending on peptide. Peptide immobilized on the liposome surface; CPSVDGAQKAGGLHH (open circle), CPSG$(R)_8$GDV (solid circle), CPSG$(R)_8$RGD (solid triangle), CPSRGDF (open triangle), CPSG$(R)_8$PPQ (solid square). Peptide surface density was 2.0×10^4 molecules/particle.

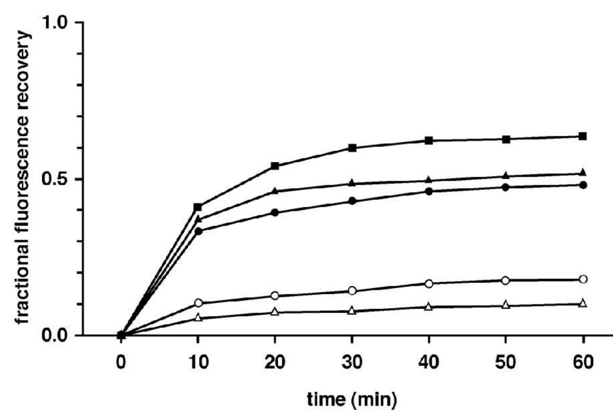

Fig. 3. Lipid-mixing of liposomes with activated platelets depending on peptide. Peptide immobilized on the liposome surface is the same as shown in Fig. 2. Reduction in energy transfer between NBD-labeled lipid and Rhodamine-labeled lipid in liposome membrane accompanied by diffusion of two probes from liposomes to platelets was monitored.