Enhanced cell binding using liposomes containing an artificial carbohydrate-binding receptor

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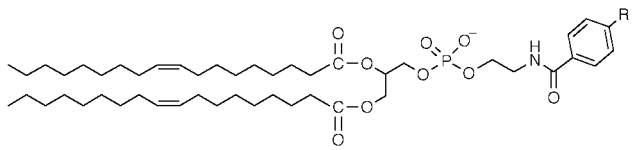
Liposomes containing phospholipids bearing a sugar-binding boronic acid group exhibit enhanced binding to erythrocyte cells.

There is a need to develop liposomes that selectively self-assemble,[1] or interact with specific cell-types,[2] as this will likely lead to improved delivery systems and imaging agents.[3,4] The standard approach is to embed in the liposome membrane high molecular weight, biotic receptors (*e.g.* lectins) that have a selective affinity for cell-surface ligands, or conversely, to coat the liposome exterior with small ligands (*e.g.* folate) that bind selectively to receptor-containing cells.[5] If there is no natural binding partner for a desired cell-surface target then the only alternative is to construct an immunoliposome,[6] or perhaps a liposome coated with a rationally-designed synthetic receptor.†

Here we evaluate the cell-binding ability of a liposome system that contains phospholipids bearing a boronic acid. Over the last ten years a large number of boronic acid host compounds have been prepared and evaluated as synthetic saccharide-binding receptors.[13,14] In general, simple arylboronic acids have modest affinities ($K_a \sim 10$–10^4 M^{-1}) for saccharides containing a vicinal *cis*-diol. Since virtually all cell membranes include glycoproteins and/or glycolipids,[15,16] it is reasonable to expect that compounds containing boronic acid groups will bind to cell surfaces.[17] We find that liposomes containing the phospholipid–boronic acid conjugate DOPEBA have enhanced affinity for erythrocyte ghosts (red blood cells) as compared to related control liposomes that contain DOPEB, a structurally similar phospholipid that lacks a boronic acid residue.‡



DOPEBA (R = B(OH)$_2$)
DOPEB (R = H)

Erythrocytes are the simplest and best characterized human cells.[16] They are good models for binding and fusion studies since they cannot undergo endocytosis. Liposome-cell binding and membrane fusion were monitored using fluorescently labelled liposomes containing 0.3% of the phospholipid probe NBD-PE [*N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine] and its resonance energy transfer quencher Rh-PE [*N*-(lissamine rhodamine B sulfonyl)phosphatidylethanolamine].[19] Two measurements are listed in Table 1, the percentage of liposomes bound to the erythrocyte ghosts, and the percentage of the bound liposomes that appear to undergo lipid mixing with the erythrocyte membranes. These values were determined using a centrifugation method to separate the cell-bound liposomes from unbound liposomes.[20] NBD-PE/Rh-PE labeled liposomes (100 nmol) and erythrocyte ghosts (5×10^8 cells) were incubated for 40 min at 37 °C in 150 μl of EDTA containing MOPS buffered saline, pH 7.4, then the mixture was centrifuged at 6000 rpm. The pellet was suspended in buffer solution and liposome binding was determined from the Rh-PE fluorescence intensity in the presence of 0.2% $C_{12}E_8$ (octaethylene glycol monododecyl ether). The fluorescence of ghosts alone in the presence of 0.2% $C_{12}E_8$ was used as 0% binding while the fluorescence of a freshly prepared liposome–ghost mixture (no incubation and spin) in the presence of $C_{12}E_8$ was taken as 100% binding. The percent of cell-bound liposomes that subsequently underwent lipid mixing with the unlabelled erythrocyte ghosts was determined from the increase in NBD-PE fluorescence which was initially attributed to dilution of the NBD-PE/Rh-PE probes. The NBD-PE fluorescence for a freshly prepared liposome–ghost mixture (without incubation and spin) was measured in the presence and absence of 0.2% $C_{12}E_8$ to determine the 100 and 0% lipid mixing, respectively. The values listed in Table 1 were calculated using the formula described by Shangguan.[20]

The erythrocyte binding and subsequent lipid mixing of 4:1 DOPEBA/POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) liposomes was compared to 4:1 DOPEB/POPC liposomes, in the presence and absence of competing sodium D-glucarate or 4-carboxyphenylboronic acid.§ As described in Table 1, only 3% of the DOPEB/POPC (4:1) liposomes were bound to the erythrocytes at neutral pH, which is in agreement with another study on related liposome systems.[20] In the case of DOPEBA/POPC (4:1) liposomes, about 33% were erythrocyte-bound. Addition of 10 mM glucarate decreased this number by half, whereas addition of 10 mM 4-carboxyphenylboronic acid had no measurable effect. It appears that binding of dianionic glucarate to the surface of DOPEBA/POPC (4:1) liposomes makes them more negatively charged and decreases their affinity for the negatively charged surfaces of glycosylated erythrocyte cells. The inability of 4-carboxyphenylboronic acid to affect liposome-erythrocyte binding is likely an example of

Table 1 Binding and apparent lipid mixing of labelled liposomes with unlabelled erythrocytes

Liposomes	Additive	Liposomes bound to cells[a] (%)	Apparent lipid mixing[ab] (%)
DOPEBA/ POPC (4:1)	none	33	18
	sodium D-glucarate	17	19
	4-carboxyphenyl boronic acid	34	15
DOPEB/ POPC (4:1)	none	3	26
	sodium D-glucarate	3	25
	4-carboxyphenyl boronic acid	3	22

[a] Each value is the average of six runs, uncertainty ± 3%. [b] Percentage of cell-bound liposomes that appear to undergo lipid mixing.

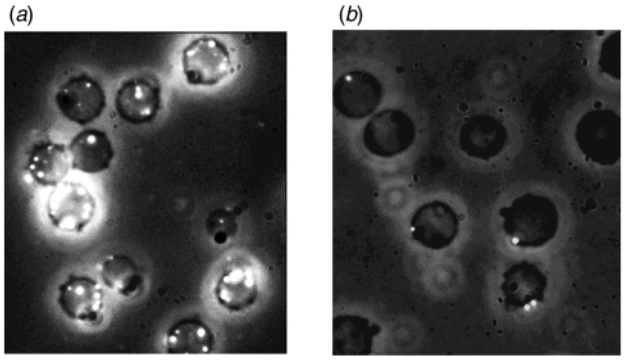


Fig. 1 Fluorescence and phase microscopy co-illumination images of Rh-PE containing liposomes bound to erythrocyte ghosts after a 10 min incubation followed by four wash cycles. The large dark circles are erythrocyte ghosts and the bright fluorescent dots are labelled liposomes. (*a*) DOPEBA/POPC (4:1) liposomes; (*b*) DOPEB/POPC (4:1) liposomes.

multivalency, where the binding constant for a monovalent boronic acid compound to the erythrocyte surface is quite weak, but the binding of a liposome that presents a partially organized array of boronic acid groups is much stronger. As expected, addition of glucarate or 4-carboxyphenylboronic acid had no effect on the binding of control DOPEB/POPC (4:1) liposomes (Table 1).

About 18% of the erythrocyte-bound DOPEBA/POPC (4:1) liposomes and 24% of the bound DOPEB/POPC (4:1) liposomes appeared to undergo lipid mixing (Table 1). Since the NBD-PE/Rh-PE lipid mixing assay is known to be susceptible to artefacts,[23,24] we decided to confirm binding and lipid mixing using a more direct method, namely, fluorescence microscopy.[20,25] In agreement with the binding data listed in Table 1, significantly more DOPEBA/POPC (4:1) liposomes were bound to the erythrocyte cells than control DOPEB/POPC (4:1) liposomes (Fig. 1). However, the fluorescence imaging showed no evidence for any lipid mixing (*i.e.* no evidence for the transfer of NBD-PE or Rh-PE probes from the liposomes to the erythrocytes),[20] which contrasts with the bulk solution data (Table 1). It appears that the increase in NBD-PE fluorescence seen in the bulk solution assay is not due to lipid mixing but is an artefact most likely due to liposome binding to the glycosylated structures on the cell surface. This result demonstrates the importance of using an alternative method to confirm the conclusions derived from lipid mixing assays.[23,24] Finally, we note that fluorescence imaging studies using liposomes filled with the water soluble dye, sulforhodamine B, produced no detectable evidence for enhanced delivery of liposomal contents.

Overall, the data is rationalized in terms of the following binding model. Unlike the DOPEB-containing liposomes, the DOPEBA-containing liposomes are able to bind strongly to the glycocalyx that projects from the erythrocyte cellular membrane. However, the DOPEBA-containing liposomes are still a significant distance from the erythrocyte membrane surface and there is no decrease in the energetic barrier to liposome–erythrocyte membrane adhesion and subsequent fusion.

The development of surface functionalized liposomes that bind to specific target cells appears to be feasible using current supramolecular technology.[2,7–12] A more difficult undertaking is the invention of structures that induce membrane fusion and thus facilitate drug delivery. Our initial efforts to address this problem will be published shortly.[26] This work was supported by the National Institutes of Health and the University of Notre Dame (Molecular Biosciences fellowship to Y. R. V.).

Notes and references

† There appears to be no previous report of enhanced cell-binding using a liposome coated with a rationally designed synthetic receptor. However, there are examples of artificial receptors enhancing small molecule recognition (ref. 7), liposome aggregation (ref. 8), and the binding and fusion of two different liposome populations (ref. 9). Also, the molecular recognition of monolayer surfaces using abiotic receptors and abiotic ligands is an active research area (refs. 10–12).

‡ The synthesis of DOPEBA has been reported before (ref. 18). DOPEB was prepared by treating dioleoylphosphatidylethanolamine with benzoyl cyanide. *Selected data* for DOPEB: δ ($CDCl_3$) 8.05 (s, 1H, N*H*), 7.86 (d, 2H, Ar*H*, *J* 7.2), 7.29–7.38 (m, 3H, Ar*H*), 5.27–5.39 (m, 4H, 2C*H*=C*H*), 5.13 (m, 1H, $POCH_2CH$), 4.27 (d, 1H, $POCH_2$), 3.83–4.04 (m, 5H, CH_2O-$POCH_2CHCH_2$), 3.60 (m, 2H, $NHCH_2$), 2.18 (t, 4H, $2COCH_2$, *J* 7.5), 2.00 (m, 8H, $2CH_2CH{=}CHCH_2$), 1.49 (m, 4H, $2COCH_2CH_2$), 1.26 [s br, 40H, $2COCH_2CH_2(CH_2)_4$ and $2(CH_2)_6CH_3$], 0.87 (t, 6H, $2CH_3$, *J* 6.6); HRMS (FAB) calc. for $C_{48}H_{81}O_9PNNa_2$ 892.5444, found 892.5475.

§ The liposomes were prepared by extrusion through polycarbonate filters as previously described (ref. 18). In each case, unilamellar vesicles (~100 nm diameter) were formed as determined by dynamic light scattering, encapsulation of aqueous contents, and lamellarity assays (ref. 21). Control experiments with liposomes containing NBD-PE/Rh-PE showed that the liposomes do not undergo significant morphology changes in the presence of sodium D-glucarate or 4-carboxyphenylboronic acid. This is in contrast to results obtained with non-liposomal aggregates of neutral and cationic amphiphiles with appended boronic acids (ref. 22).

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