Interactions between Cationic Liposomes and Drugs or Biomolecules

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ABSTRACT

Multiple uses for synthetic cationic liposomes composed of dioctadecyldimethylammonium bromide (DODAB) bilayer vesicles are presented. Drugs or biomolecules can be solubilized or incorporated in the cationic bilayers. The cationic liposomes themselves can act as antimicrobial agents causing death of bacteria and fungi at concentrations that barely affect mammalian cells in culture. Silica particles or polystyrene microspheres can be functionalized by coverage with DODAB bilayers or phospholipid monolayers. Negatively charged antigenic proteins can be carried by the cationic liposomes which generate a remarkable immunoadjuvant action. Nucleotides or DNA can be physically adsorbed to the cationic liposomes to be transferred to mammalian cells for gene therapy. An overview of the interactions between DODAB vesicles and some biomolecules or drugs clearly points out their versatility for useful applications in a near future.

Key words: interactions, dioctadecyldimethylammonium bromide, phospholipids, liposomes, drugs, surfaces.

INTRODUCTION

Since their introduction as bilayer-forming synthetic compounds (Fendler 1980), dihexadecylphosphate (DHP) or dioctadecyldimethylammonium (DODA) salts have found many different uses in strategic applied areas (Carmona-Ribeiro 1992). In particular, synthetic cationic liposomes have been sucessfully employed to interact with negatively charged surfaces or biomolecules such as prokaryotic (Martins *et al*. 1997) or eukaryotic cells (Carmona-Ribeiro *et al*. 1997), antigenic proteins (Tsuruta *et al*. 1997), nucleic acids (Behr 1993), synthetic polymers and latex (Carmona-Ribeiro & Midmore 1992, Lessa & Carmona-Ribeiro 1996) and mineral surfaces (Rapuano & Carmona-Ribeiro 1997). In this work, some of their most recent uses in several applied areas are shown.

In Figure 1, the several possibilities for closed bilayer vesicles as drug carriers are illustrated. A water soluble molecule (S) can be carried inside the water vesicle compartment, an hydrophobic molecule (H) can be solubilized in the hydrophobic moiety of the bilayer and an amphiphilic drug (T) can be partitioned between the bilayer and the water phase (Figure 1). Supported bilayers (Carmona-Ribeiro & Midmore 1992, Rapuano & Carmona-Ribeiro 1997) or monolayers (Carmona-Ribeiro & Herrington 1993) have been described using silica or polystyrene particles (latex) (Figure 1). The hydrophobic/hydrophilic nature and the presence of electric charge on the solid sup-

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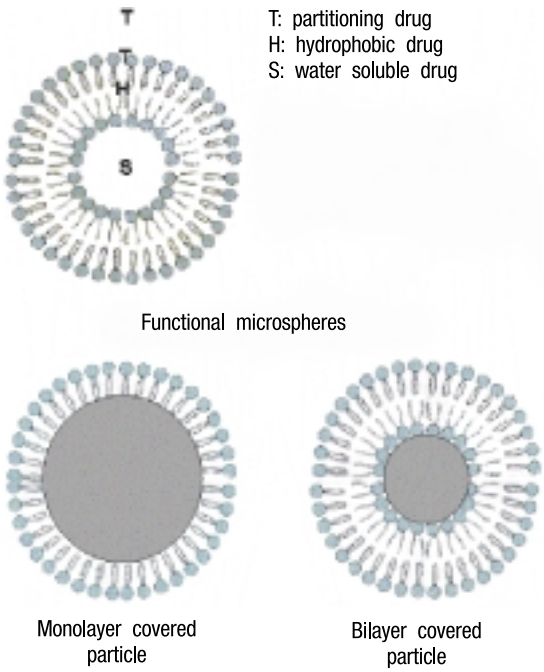

Fig. 1 – Bilayer vesicles and liposomes as drug carriers or as sources of organized supramolecular assemblies to cover solid particles.

porting particle have a major role in determining the lipidic supramolecular assembly obtained at the solid surface. The negatively charged nature of sulfate polystyrene microspheres leads to deposition of cationic bilayers onto the surface and vice-versa (Carmona-Ribeiro & Midmore 1992) whereas the neutral character of phosphatidylcholine (PC) or dipalmytoylphosphatidylcholine (DPPC) leads to monolayer coverage of amidine latex with the phospholipid polar heads uppermost and the lipid hydrocarbon tails hydrophobically interacting with the latex surface (Carmona-Ribeiro & Herrington 1993).

The molar absorptivity for Amphotericin B, an hydrophobic drug currently used as a potent fungicide, was determined in small DODA bromide (DODAB) vesicles (1.5 mM DODAB) as solubilizers. It is 160,406 M^{-1} cm^{-1}. This shows that these cationic vesicles are excelent solubilizers for the drug since the molar absorptivity for the drug in its best solvent mixture (methanol/dimethylsulfoxide 1:1) is 164,300 M^{-1} cm^{-1}. Formulation of hydrophobic drugs will possibly be substantially improved by using DODAB vesicles as solubilizers. However, prospective DODAB uses cannot avoid a systematic evaluation of diferential toxicity for these cationic liposomes.

Table I illustrates the compared toxicity of DODAB liposomes for cultured mammalian cells and some bacteria or fungi. Mammalian cells are more resistant to DODAB than are bacteria or fungi remaining 50% viable at 1.0*mM* DODAB whereas, for the latter, 50% of viability occurs over the *micromolar* range of DODAB concentrations. Mammalian cells remain ca. 100% alive at DODAB concentrations where bacteria and fungi do not survive. DODAB liposomes are prone to further uses as bactericides themselves in which multiple drugs against multiple infections can be included.

Determination of adsorption isotherms for lipids from liposomes onto latex, silica or bacteria followed by linearization of the langmuirian curves allows calculations of parameters as affinity constants and adsorption maxima (Jackson *et al*. 1986), which are valuable parameters to ascertain the product of the liposome/surface interaction (Table II). The occurrence of opposite charge for the liposome and surface pair generally drives deposition of one bilayer onto the latex or the silica particle. Examples for this are the interactions between large or small DODAB vesicles and latex where the cationic DODAB deposits as bilayers onto the negatively charged sulfate polystyrene particles. Similarly, interactions between small or large DHP vesicles and oppositely charged amidine polystyrene particles leads to deposition of DHP bilayers onto the latex (Table II). However, the negatively charged *E. coli* cell does not interact with cationic DODAB or DODAC vesicles leading to bilayer deposition. There is mere adhesion of entire vesicles at the cell surface. Given the roughness and complexity of the supramolecular assembly characteristic of the bacteria cell wall, which includes protuding sugars from glicolipids and lipoproteins, complex protein structures with multiple functions as recognition,

TABLE I

Diferential cytotoxicity of cationic DODAB liposomes. Interaction time between small DODAB vesicles and cells was fixed at 1 h. Adapted from Carmona-Ribeiro *et al*. 1997, Campanhã *et al*. 1999.

Cell type	Viable cells concentration (cells/mL)	DODAB concentration for 50% survival (mM)
Normal Balb-c 3T3 (clone A31) mouse fibroblasts	10^4	1.000
SV40-transformed SVT2 mouse fibroblasts	10^4	1.000
C. albicans	2×10^6	0.010
E. coli	2×10^7	0.028
S. typhimurium	2×10^7	0.010
P. aeruginosa	3×10^7	0.005
S. aureus	3×10^7	0.006

adhesion, transport, etc, it is not straightforward to achieve a simple explanation for the absence of cationic vesicle disruption at the cell surface. Neutral phospholipids such as PC or DPPC from vesicles deposits as an odd number of monolayers onto the amidine latex surface. This agrees with the interpretation of a first monolayer coverage on the solid surface with the phospholipid polar heads uppermost. In this case, adsorption would be basically driven by the hydrophobic attraction between the lipid hydrocarbon tails and the hydrophobic surface.

Table III shows how the cationic DODAB liposomes are useful to carry oppositelly charged biomolecules such as proteins or a nucleotide, 2'-deoxyadenosine 5'-monophosphate. The cationic liposomes are useful as immunoadjuvants for induction of delayed type hypersensitivity towards the antigenic 18 kDa heat-shock protein of *M. leprae*. Incorporation of the protein in the adjuvant liposomes increases the cellular immunoresponse ca. 10 times (Tsuruta *et al*. 1997). Besides the electrostatic attraction driving incorporation of the protein onto the liposome, there is also the hydrophobic attraction between the liposome and the protein that accounts for the large incorporation measured at higher salt concentrations (Table III). Bovine serum albumin (BSA) and anti-BSA incorporation onto the cationic liposomes is more sensitive to ionic strength than is incorporation of hsp −18 kDa from *M. Leprae* (Table III). Incorporation substantially decreases when performed in PBS instead of water (Table III) showing the relative importance of electrostatics vs. hydrophobic forces determining protein incorporation in the liposome. The nucleotide in Table III is a typical case of partitioning molecule whose incorporation in the cationic bilayer requires the large electrostatic attraction that occurs in pure water at zero ionic strength.

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TABLE II

Affinities (K) and maximal adsorption (x_{max}) for different liposome-forming amphiphiles onto a variety of organic, inorganic or biological surfaces. Models for organic synthetic surfaces are different types of latex (polystyrene) particles; for inorganic surfaces, conventional hydrophilic silica (Aerosil OX-50) and, for biological surfaces, *E. coli* cell surfaces. Liposomes were obtained from the following lipids: phosphatidylcholine (PC), dipalmytoilphosphatidylcholine (DPPC), dioctadecyldimethylammonium bromide (DODAB) or chloride (DODAC), sodium dihexadecylphosphate (DHP) or monosialoganglioside (GM1). Latex particles are quoted as sulfate polystyrene (SP) or amidine polystyrene (AP) followed by the mean latex diameter in nm. Silica particles are Aerosil OX-50 (50 nm mean diameter) from Degussa. Adapted from Tápias *et al*. 1994, Campanhã *et al*. 1999, Carmona-Ribeiro & Midmore 1992, Carmona-Ribeiro & Herrington 1993, Sicchierolli & Carmona-Ribeiro 1995, Sicchierolli *et al*. 1995, Rapuano & Carmona-Ribeiro 1997.

Liposome	Particle	Affinity constant, K (M^{-1})	Adsorption maxima, x_{max} (molecules per m^2)	Lipid assembly at the surface
DODAB/SV	*E. coli* cell	0.14	200×10^{17}	Entire small vesicles adhered to the cell wall
DODAC/LV	*E. coli* cell	45.20	345×10^{17}	Large vesicles adhered to the cell wall
DODAB/SV	SP100	35.60×10^4	36×10^{17}	Bilayer
DODAB/LV	SP277	2.56×10^4	43×10^{17}	Bilayer
DODAC/LV	SP285	29.00×10^4	35×10^{17}	Bilayer
DHP/LV	AP850	75.00×10^4	53×10^{17}	Multibilayers
DHP/SV	AP97	51.00×10^4	55×10^{17}	Bilayer
PC/SV	AP104	50.00	14.5×10^{17}	Monolayer
		110.00	47.0×10^{17}	Monolayer+Bilayer
DPPC/SV	AP104	56.00	20.0×10^{17}	Monolayer
		-	63.6×10^{17}	Monolayer+Bilayer
DODAB/SV	Silica50	63.70×10^4	$34 - 70 \times 10^{17}$	Bilayer+adhered vesicles
GM1 micelles	PC Mono+Bilayer Covered AP172	-	1.7×10^{17}	1 GM1 per 8 PC molecules forming a layer of adjacent sugar polar heads at the bilayer/water interface

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TABLE III
Incorporation (%) of a variety of biomolecules onto cationic DODAB liposomes.
Adapted from Tsuruta *et al.* 1997, Carvalho & Carmona-Ribeiro 1998, Kikuchi *et al.* 1999.

Liposome type and concentration	Biomolecule	Medium	Incorporation (%)
DODAB LV/ 2.6 mM	hsp 18 kDa (*M. leprae*)	water	94-100
		PBS	67-74
DODAB LV/ 4.0 mM	BSA	water	100
		PBS	32-35
	anti-BSA	water	96-98
		PBS	26-31
DODAB LV/ 0.5 mM	2'-deoxyadenosine 5'-monophosphate	water	22 - 25
DODAB LV/ 0.5 mM		5mM NaBr	2 - 4

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