Biomimetic Particles

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Summary: Electrostatically driven bilayer coverage from bilayer fragments onto oppositely charged latex produces a highly homodisperse particulate for biomolecules adsorption. Polystyrene sulfate (PSS) particles were covered with single cationic dioctadecyldimethylammonium bromide (DODAB) bilayers. Biomolecules adsorbed were DNA, bovine serum albumin (BSA), cholera toxin (CT) and a mixture of purified 18 kDa/14 kDa *Taenia crassiceps* proteins (18/14-*Tcra*). Firstly, the DODAB/PSS assembly was characterized at 1 mM NaCl and 5×10^9 PSS particles/mL over a range of DODAB concentrations (0.001 -1 mM) by means of dynamic light scattering for particle sizing and zeta-potential analysis. 0.01 mM DODAB was enough to produce perfectly homodisperse and cationic bilayer-covered particles. Secondly, under these experimental conditions, BSA, CT, and 18/14-*Tcra* adsorbed onto biomimetic bilayer-covered particles yielding langmuirian isotherms with 1.23×10^{17}, 0.82×10^{17}, 3.37×10^{17} molecules per m^2 at limiting adsorption and affinity constants (K) of 3.17×10^{10}, 3.39×10^{10}, and 1.75×10^{10} M^{-1}, respectively. For DNA adsorption, isotherms did not attain an adsorption maximum up to 20 micrograms/mL DNA. PSS/DODAB/biomolecule assemblies were also characterized by means of dynamic light scattering and zeta-potential analysis yielding highly monodisperse particles at and above maximal adsorption. In summary cationic bilayer-covered particles are a novel, highly organized and, possibly, general support for biomolecules immobilization.

Keywords: biomolecule adsorption; bovine serum albumin; cholera toxin; dioctadecyldimethylammonium bromide; DNA λ phage; immobilization; lipid-coverage; polymeric particles; *Taenia crassiceps* proteins

Introduction

Particles are finding a large variety of biomedical and pharmaceutical applications since their size scale can be similar to that of biological molecules (e.g., proteins, DNA) and structures (e.g., viruses and bacteria). They are currently being used in imaging,[1] biosensing,[2] gene and drug delivery,[3] and vaccines[4]. On the other hand, cationic lipids electrostatically combine with a vast variety of negatively charged biomolecules or biological structures.[5] Silica,[6] latex[7] or hydrophobic drug particles[8] have been coated by cationic lipids and characterized by means of adsorption isotherms, mean particle size from dynamic light scattering, surface potential analysis and colloidal stability. Lately, colloid stability and optimization of cationic bilayer deposition on particles has been achieved from systematic studies determining effects of particle-lipid interacting pair and intervening medium properties such as ionic strength.[9] In this work, polystyrene sulfate (PSS) nanoparticles with 301 nm mean diameter are covered with a dioctadecyldimethylammonium bromide (DODAB) bilayer from DODAB bilayer fragments (BF)[10] and used for adsorption and immobilization of biomolecules.

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Experimental Procedures

Lipids, Polystyrene Nanospheres, Biomolecules

Dioctadecyldimethylammonium bromide (DODAB) 99,9% pure was obtained from Sigma-Aldrich (St Louis, MO, USA). Anionic polystyrene sulfate (PSS) particles (Lot 10-66-58), nominal mean diameter of $301 \pm 2\%$, 188,944 cm^2g^{-1} specific surface area, surface charge density of 1.68 $\mu C\,cm^{-2}$ and -64 ± 2 mV mean zeta-potential (Table 1) were purchased from Interfacial Dynamics Corporation (Portland, Or, USA) and a stock suspension containing 2×10^{10} particles/mL was prepared in 1 mM NaCl, which is an adequate ionic strength to assemble DODAB BF as a single bilayer onto particles.[9] DNA phage λ (λ-DNA) was purchased from Sigma-Aldrich at initial concentration of 565 μg/mL and diluted at final concentrations of 0.5 at 20 μg/mL; bovine serum albumin (BSA) and cholera toxin (CT) were purchased from Sigma-Aldrich, and prepared as a 1 mg/mL stock solution in NaCl 1 mM and stored in a freezer in 1 mL aliquots for quick use. Purified fractions of native 18- and 14-kDa *T. crassiceps* proteins (18/14-*Tcra*) were prepared as previously described[11] and diluted 1:20 in 0.15 M NaCl to obtain a stock solution at 1.4 mg/mL. Protein concentration was determined by the Bio-Rad Protein microassay, based on the method of Bradford (reagent cat# 500-0006, Bio-Rad, Hercules, CA, USA), using a standard curve (5–35 μg/mL) of BSA. NaCl and all other reagents were analytical grade. Water was Milli-Q quality.

Preparation of Lipids Dispersions and Analytical Determination of Lipid Concentration

Small DODAB bilayer fragments (BF), 81 ± 1 nm mean diameter and 45 ± 2 mV mean zeta-potential (Table 1), were prepared by sonication with titanium macrotip probe in 1 mM NaCl Milli-Q water solution at ca. 2.0 mM DODAB as previously described.[12] Analytical concentrations of DODAB or NaCl were determined by halide microtitration.[13]

Preparation of PSS/DODAB and PSS/DODAB/Biomolecules

PSS particles (2×10^{10}/mL) and DODAB lipid BF (2.0 mM) were always prepared in 1 mM NaCl and diluted to the final desired concentration using this same salt solution. PSS (5×10^9 particles/mL) and DODAB BF dispersions (1×10^{-4}–1 mM) interacted for 1 h/25 °C. DODAB final concentration for producing the assemblies was selected

Table 1.
Physical properties of particles, DODAB dispersion, DODAB-covered particles at maximal coverage, proteins and proteins/DODAB-covered particles at 1 mM NaCl.

Sample[a)]	DDA (mM)	Protein (μg/mL)	Mean diameter (nm)	Zeta-Potential (mV)	Polydispersity index
PSS[b)]	–	–	301 ± 2	-60 ± 1	0.064 ± 0.020
DODAB	2,000	–	81 ± 1	45 ± 2	0.230 ± 0.006
PSS/DODAB	0.01	–	309 ± 2	48 ± 2	0.040 ± 0.010
DODAB/λ DNA	0.01	2.5	978 ± 52	19 ± 2	0.316 ± 0.03
PSS/DODAB/λ DNA*	0.01	2.5	332 ± 2	43 ± 1	0.043 ± 0.021
PSS/DODAB/λ DNA	0.01	3.5	424 ± 5	-49 ± 2	0.111 ± 0.029
DODAB/18/14-*Tcra*	0.01	25	295 ± 3	6 ± 6	0.167 ± 0.023
PSS/DODAB/18/14-*Tcra*	0.01	25	328 ± 3	11 ± 8	0.060 ± 0.020
DODAB/CT	0.01	25	2384 ± 203	14 ± 3	0.519 ± 0.013
PSS/DODAB/CT	0.01	25	344 ± 4	16 ± 2	0.08 ± 0.03
DODAB/BSA	0.01	25	113 ± 8	-31 ± 1	0.362 ± 0.021
PSS/DODAB/BSA	0.01	25	320 ± 3	-35 ± 2	0.041 ± 0.014

a) Dispersions in 1 mM NaCl.
b) The particle number density was 5×10^9 particles/mL; PSS particle mean diameter from transmission electron microscopy, given by the supplier, is 301 ± 2 nm.

as 0.01 mM at 5×10^9 PSS particles per mL since this concentration is the one required to cover each nanosphere with a DODAB bilayer. In a second experimental step, biomolecules were added to the PSS/DODAB mixture at final concentrations ranging from 1 to 50 μg/mL for proteins and 0.5 to 20 μg/mL for λ-DNA, for 1 h/25 °C interaction. Thereafter, sizes, zeta-potentials, and polydispersities were determined. Details on particle number densities, DODAB concentrations and/or biomolecules concentrations are quoted on Table 1. Considering the PSS total area in the experimental condition above (2.84×10^{15} ηm^2), the selected DODAB concentration (0.01 mM) was precisely sufficient to produce bilayer-covered nanospheres and DODAB is not expected to be found free in dispersion.

Determination of Mean Diameters, Size Distribution, Polydispersity and Zeta-Potentials for DODAB, Biomolecules, Particles Dispersions, or their Mixtures

Particle size (mean diameter *Dz*), size distribution, polydispersity and zeta-potential (ζ) in the presence or absence of PSS, DODAB or biomolecules were determined using the ZetaPlus-ZetaPotential Analyzer (Brookhaven Instruments Corporation, Holtsville, NY), which was equipped with a 677 nm laser and dynamic light-scattering (PCS) at 90° for particle sizing. Mean diameters were obtained by fitting data to log-normal size distributions which does not discriminate between one, two, or more different populations and considers always all scattering particles as belonging to one single Gaussian population. On the other hand, for the size distribution data, fitting was performed by the apparatus software using the non-negatively constrained least squares (NNLS) algorithm, which is a model independent technique allowing to achieve multimodal distributions.[14] ζ was determined from electrophoretic mobility μ in 1 mM NaCl and the Smoluchowski's equation: $\zeta = \mu\eta/\varepsilon$, where η is the medium viscosity and ε the medium dielectric constant.

Determination of Adsorption Isotherms for BSA, CT, 18/14-*Tcra* and DNA

For proteins adsorption isotherms on PSS/DODAB particles were obtained by mixing 0.05 mL of the stock PSS solution (2×10^{10} particle/mL) with 0.01 mL DODAB BF (0.2 mM) and 0.14 mL of the appropriate biomolecule dilution in NaCl 1 mM. Final concentration BSA, CT and 18/14-*Tcra* in the assay ranged from 0–50 μg/mL for BSA and CT, or 0–74 μg/mL, at fixed concentration of 5×10^9 PSS particle/mL and 0.01 mM DODAB BF. After 1 h interaction at 25 °C, a clear supernatant was obtained by centrifugation at 15,000 rpm for 1 h. The concentration of protein in the supernatant was determined by Bradford microassay using a standard curve prepared from 5–35 μg/mL BSA. A microplate reader equipped with a 595 nm filter (Ultramark, Model 550 Bio-Rad, Hercules, CA, USA) was used for absorbance measurement. The amount of adsorbed protein was determined by the difference between the total protein added and the amount of protein recovered in the supernatant.

Adsorption isotherms for λ-DNA on PSS/DDA was obtained by mixing 0.5 mL of the stock PSS solution (2×10^{10} particle/mL) with 0.01 mL DODAB BF (2 mM) and 1,490 mL of the appropriate λ-DNA dilution in NaCl 1 mM. Final concentration in the assay ranged from 0–20 μg/mL, at fixed concentration of 5×10^9 PSS particle/mL and 0.01 mM DODAB BF.

Adsorption was expressed as the number of molecules adsorbed per square meter on PSS/DODAB. Curves draw on the isotherms were fitted using cubic polynomial regression.

Results and Discussion

DODAB/PSS assembly was characterized at 1 mM NaCl and 5×10^9 PSS particles/mL over a range of DODAB concentrations (1×10^{-4}–1 mM) by means of dynamic light scattering for particle sizing and zeta-potential analysis. At 5×10^9 PSS particles/mL, 0.01 mM DODAB produced perfectly

homodisperse and cationic bilayer-covered particles (named biomimetic particles). Oppositelly charged biomolecules can therefore be driven to biomimetic particles via electrostatic attraction.

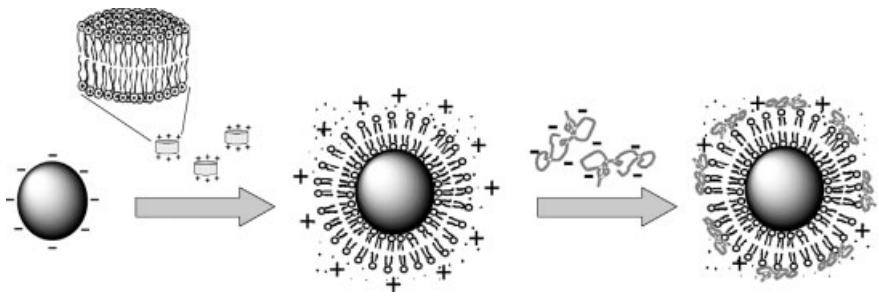
Scheme 1 shows the steps performed to obtain the model of biomimetic particles. In the first step, at 1 mM NaCl, the polystyrene sulfate (PSS) polymeric particles were covered by oppositely charged DODAB bilayer fragments (BF). This procedure was previously shown to result in one bilayer coverage surrounding each particle from determination of adsorption isotherm, electron transmission microscopy of positively stained bilayer-covered particles, increase in the mean particle diameter of 8–10 nm, reversal of particle zeta-potential from negative to positive and improved colloid stability at and above bilayer coverage.[10,12] Apparently, 1 mM ionic strength provided by 1 mM NaCl was effective in inducing a hydrophobically driven autoassociative process of adjacent bilayer fragments deposited onto the polymeric particles that did not take place in water or ionic strengths smaller than 1 mM.[9]

At charge neutralization, there is maximal interparticle aggregation and minimal colloid stability (data not shown). Further increasing DODAB concentration stabilizes the system at sizes and zeta-potentials consistent with DODAB bilayer adsorption. Table 1 shows sizes and zeta-potentials for PSS particles, DODAB BF dispersions, DODAB-covered particles, and PSS/DODAB/biomolecule assemblies. One should notice that the zeta-potential for DODAB BF is the same as the one for PSS/DODAB (Table 1). In addition, PSS particles diameter of 301 nm increases to 309 nm in the presence of 0.01 mM DODAB indicating deposition of one single, 4 nm thick DODAB bilayer on particles (Table 1). This is consistent with the zeta-potential data showing reversal of particle surface charge. Adsorption isotherms for DODAB onto 301 nm PSS particles have previously shown a limiting adsorption consistent with bilayer deposition[7] as illustrated in Scheme 1.

Deposition of bilayers onto particles from lipid bilayer vesicles that are in the rigid gel state at room temperature (such as DODAB liposomes) has not been a straightforward procedure; thus the problem has been circumvented by using bilayer fragments, which are easily obtained from macrotip ultrasonic dispersion of the lipid powder at a temperature above DODAB bilayer phase transition temperature.[10]

DNA and proteins were successfully immobilized onto biomimetic particles yielding narrow particle size distributions for the assemblies as shown in Table 1. This low polydispersity contrasts with the controls for DODAB BF alone, DODAB/DNA without particles, DODAB/protein alone (Table 1).

A large affinity was obtained for proteins adsorbing on biomimetic particles at 1 mM NaCl (Table 2). Interestingly enough, this affinity was much higher than the



Scheme 1.
Steps followed to obtain model biomimetic particles.

Table 2.
Affinity constants (K) and maximal adsorption $(x/m)_{max}$ for different antigenic proteins onto DODAB-covered PSS particles at 1 mM NaCl or, for liposome-forming DODAB onto sulfate polystyrene (PSS).

Biomolecule		Particle[a)]	Affinity constant, K (M^{-1})	Adsorption maxima, $(x/m)_{max}$ (molecules per m^2)
Protein	BSA	PSS/DODAB	3.17×10^{10}	1.23×10^{17}
	CT	PSS/DODAB	3.39×10^{10}	0.82×10^{17}
	18/14-*Tcra*	PSS/DODAB	1.75×10^{10}	3.37×10^{17}
Lipid[b)]	DODAB/SV	PSS 100	35.60×10^{4}	36×10^{17} (bilayer)
	DODAB/LV	PSS 277	2.56×10^{4}	43×10^{17} (bilayer)
	DODAC/LV	PSS 285	29.00×10^{4}	35×10^{17} (bilayer)

[a)] For biomolecules, PSS particle mean diameter from transmission electron microscopy, given by the supplier, is 301 ± 2 nm. For liposome, PSS particle are quoted as PSS followed by the mean latex diameter in mm.
[b)] Values taken from Carmona-Ribeiro, 2000.[15]

affinity exhibited by the DODAB bilayer for the polymeric particles.[15] This large protein affinity for cationic supported bilayer was previously observed also for BSA or immunoglobulins adsorbing in pure water onto DODAB large vesicles.[15] The maximal adsorption values that are shown in Table 2 were calculated from the Langmuir model indicating a limiting adsorption that is 10 times smaller for proteins than for lipids, consistently with the much lower molecular weight of the lipids as compared with the proteins (Table 2).

DNA adsorbed onto the oppositely charged biomimetic particles yielding an adsorption isotherm of the linear type, ie, no limiting adsorption could be attained over the 0–20 micrograms/mL range of DNA concentrations (not shown). Nevertheless, about 100 % adsorption of DNA on particles was achieved over this concentration range. As expected for a polyelectrolyte, polydispersity was not so low as was the polydispersity for PSS/DODAB/protein assemblies obtained after charge reversal (Table 1). Possibly, Dna immobilization caused a certain extent of bridging flocculation and a broader range of size distributions than those for immobilized proteins.

Conclusion

Polystyrene sulfate nanoparticles covered with one bilayer of cationic lipid (dioctadecyldimethylammonium bromide) adsorb proteins producing a highly ordered, effective and monodisperse particulate with controllable particle size, shape and dispersity. Eventually, for DNA immobilization, polydispersity could be further optimized by systematic evaluation of experimental conditions. An additional advantage of the PSS/DODAB assembly is that the size of the particle composed of lipid surface and polymeric particles core can be easily controlled by using the system described in this paper. Cationic bilayer-covered polymeric particles are a novel, highly organized and general support for biomolecules immobilization.

Acknowledgements: Financial support from FAPESP and CNPq are gratefully acknowledged. N. L. is the recipient of a postdoctoral FAPESP fellowship and H.R. is the recipient of a MSc CNPq fellowship.

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