Research Paper

Bovine serum albumin interactions with cationic surfactant vesicles decorated by a low-molar-mass polysaccharide

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ABSTRACT

The complexation of bovine serum albumin (BSA) with bare and sodium-hyaluronate (Na-HA)-modified didodecyl(dimethyl)ammonium bromide (DDAB) vesicles is systematically investigated by static and dynamic light scattering in a broad range of scattering angles. The aggregation number of DDAB vesicles increases as a function of added Na-HA concentration because of the attractive interactions between the cationic surfactant and anionic polysaccharide that reduce the repulsions between the charged head groups of the surfactant. This is confirmed by the inversion of the vesicular surface charge upon addition of Na-HA. Interactions of BSA with DDAB vesicles are surprisingly found to be much stronger when Na-HA is adsorbed on the vesicles although in this case protein and vesicular surface charge are of the same sign. Apparently the anisotropically charged BSA globules are more effectively anchored on the complex interface of the Na-HA decorated DDAB vesicles in comparison to the uniform positively charged interface of bare DDAB vesicles. In addition the strong associations of the globular protein with the vesicles compromise the protein's secondary structure. This system can be a useful template for versatile nanocapsules with the ability to bind substances via hydrophobic and electrostatic interactions.

1. Introduction

Self-assembly at the nanoscale is widely used for the formation of functional nanomaterials. Recent examples include hierarchical composite materials [1] and dual nanodrugs [2]. Nanocarriers based on self-assembled surfactants and lipids gather increased interest for applications on delivering bioactive substances as enzymes, drugs, toxins and genetic material [3]. Formation of closed bilayer membranes i.e. surfactant vesicles or liposomes offers the advantage of high loading capacity and the ability to encapsulate both hydrophobic and hydrophilic entities. Vesicular self-assemblies containing biomolecules as proteins and DNA have the inherent properties of versatility and biocompatibility and have shown promising performance as carriers of hydrophobic drugs against cancer cells [4]. On the other hand interactions of globular proteins with lipid bilayers have been proved to cause structural changes on the interfaces with implications to the protein-cell interactions [5]. These examples can be viewed under the more general and advancing emerging field of nanoarchitectonics for the design and fabrication of dynamic functional nanomaterials [6].

Interactions of proteins with polyelectrolytes is a subject of intense research during the last two decades because of their versatile applications as in protein delivery and separation, tissue engineering and wound healing [7]. Self-assembly of block polyelectrolyte micelles with proteins are proved to affect the inter-micellar hierarchical
organization [8] while in the case of thermoresponsive micellar corona the presence of proteins interferes with temperature response of the nano-formulations [9]. The anisotropic charge patch on a protein surface is considered responsible for the complex nature of the attraction with charged macromolecules. Complexation of polyelectrolytes with liposomes have been investigated as a means to mimic cell membranes, stabilize liposomes or improve drug delivery efficiency [10–12]. Under these considerations the investigation of the adsorption of proteins on polyelectrolyte-modified charged vesicles will provide insights to the interactions between objects that contain both signs of charges and hydrophobic domains and opens many possibilities for more efficient and controlled loading.

Recently we illustrated that the solution properties of xanthan (a high molar mass polysaccharide) can be controlled by the interactions with the cationic surfactant dodecyltrimethylammonium bromide (DTMAB). In that case electrostatic attachment on the polysaccharide chains induced a hydrophobic character to the natural hydrophilic polyelectrolyte [13]. In this study vesicles of the cationic surfactant DDAB are decorated by an oppositely charged polysaccharide through the adsorption of low molecular weight Na-HA. This appears to offer a flexible route for tuning the surface charge and aggregation number (number of surfactant molecules) of the vesicles. The resulting decorated vesicles potentially embody a composite landscape for electrostatic and hydrophobic interactions with proteins. Indeed the interactions with BSA are much stronger when the vesicles are decorated with Na-HA and this can be related to the change in protein’s secondary structure. This work demonstrates that charged bilayer-forming systems can be tunable nanocarriers of bioactive compounds by modification with polyelectrolytes and can be used as templates for the study of association of globular proteins with soft complex nanostructures.

2. Material and methods

2.1. Materials and samples preparation

Didodecyldimethylammonium bromide (DDAB) was purchased from Fluka and used without further purification. Hyaluronic acid in the sodium salt form (Na-HA) with M = 5000 g/mol (PDI ~ 1.5) was a kind gift from Uni-Pharma (Greece). Bovine serum albumin (BSA) was purchased from Sigma-Aldrich and was used without further treatment. Stock aqueous solutions from the separate components i.e. DDAB (1 mg/ml), Na-HA (0.3 mg/ml) and BSA (3 mg/ml) were prepared at 0.01 M NaCl and pH 7 and kept overnight to dissolve and equilibrate at 4 °C. The salt concentration was set by adding the desired volume of NaCl (1 M). The final volume ratios of the mixtures were obtained by mixing proper volumes of stock solutions and adding distilled water with the same salt concentration and pH as the stock solution under gentle stirring. Water was first mixed with the salt solution and DDAB solution was subsequently added. Na-HA or BSA stock solutions were added after DDAB for preparation of dual mixtures. In ternary mixtures Na-HA solution was added before BSA. All experiments were performed at room temperature.

2.2. Light scattering

An ALV-CG-3 goniometer system with an ALV-5000/EPP multi tau digital correlator and a He-Ne laser with wavelength of \( \lambda = 632.8 \) nm were used for the light scattering (LS) measurements. Static (SLS) and dynamic (DLS) light scattering data were collected over a wide angular range from \( \theta = 30^\circ \) to \( \theta = 130^\circ \).

In SLS the time-averaged scattered intensity as a function of scattering angle is reduced to the Rayleigh ratio \( R(q) \) by standard methods [14] and the weight average molecular weight \( M \) and form factor \( P(q) \) of the scattering particles can be extracted from Eq. (1).

\[
\frac{Kc}{R(q)} = \frac{1}{MP(q)} \tag{1}
\]

where \( c \) is the particle concentration in solution, \( q \) the scattering wave vector given by \( q = \frac{4\pi n_0}{\lambda} \sin \theta/2 \) and \( K \) a prefactor given by \( K = \frac{\pi^2 q^4}{6} \left( \frac{\partial n_0}{\partial c} \right)^2 \). Where \( n_0 \) is the solvent’s refractive index and \( \partial n_0/\partial c \) the refractive index increment of the scattering particles in the specific solvent.

\[
\frac{R(q)}{Kc} = Me^{-\frac{1}{2}q^2h_0^2} + c(q^2)^2 \tag{2}
\]

In the Guinier approximation \( P(q) = e^{-\frac{1}{2}q^2h_0^2} \) the radius of gyration \( R_g \) of the scattering particles is obtained. In our case the SLS data appeared nonlinear in the Guinier representation (see Results and Discussion) and hence the molecular mass and radius of gyration were calculated by fitting with Eq. (2). A quadratic term in \( q^2 \) was included in order for an accurate extrapolation to low \( q \) to be performed.

In DLS the exported scattered light intensity autocorrelation functions \( g_1(\tau) \) are connected [15] to the field autocorrelation functions \( g_r(\tau) \) using the Siegel relation \( g_r(\tau) = 1 + \beta \Gamma_r(\tau) \) where \( \beta \) is a normalization constant and \( \tau \) the lag-time. Field autocorrelation functions were treated by cumulant analysis and the characteristic relaxation rate \( \Gamma(\tau) \) was extracted from the main relaxation time \( \tau_{rel}(q) \) by \( \Gamma(\tau) = 1/\tau_{rel}(q) \).

\[
\Gamma(\tau) = Dq^2 + C(q^2)^2 \tag{3}
\]

A second order approximation in \( q^2 \) (Eq. (3)) led to the diffusion coefficient \( D \) and the hydrodynamic radius \( R_h \) was calculated by the Stokes-Einstein relation (Eq. (4)) where \( n \) is the solvent viscosity.

\[
R_h = \frac{K_b T}{6\pi n D} \tag{4}
\]

In all the DLS experiments a single dynamic mode (CONTIN analysis) was found for all scattering angles. This justifies the use of cumulants which provides equivalent results with the relaxation time obtained at the maximum of the CONTIN distribution functions.

2.3. Electrophoretic light scattering

Electrophoretic light scattering (ELS) was performed on a Zetasizer Nano-ZS by Malvern Instruments Ltd. Zeta potential calculation was made by the Henry equation in the Smoluchowski approximation. The \( \zeta \) values reported are averages of 10–20 measurements taken at 173° angle. All the experiments were performed at room temperature.

2.4. Circular dichroism

Circular dichroism (CD) measurements were carried out on a Jasco J-815 CD spectrophotometer with a peltier model PTC-423S/15 thermo stabilizing system. The solutions were loaded on 1-mm quartz Suprasil cells. In the aqueous mixtures BSA concentration was at 0.15 mg/ml which was the optimum value for adequate CD signal and relevance with the conditions of LS experiments. The contents of \( \alpha \)-helix, \( \beta \)-sheet and random coil were estimated by K2D3 software [16].

3. Results and discussion

3.1. Complexation of DDAB with Na-HA

DDAB is a double-chained cationic surfactant with a roughly cylindrical shape and a packing parameter about 0.62 that drive it to create bilayer structures in water [17]. Cryo-TEM investigations have clearly shown the formation of single-wall, double-wall and multi-compartment vesicles in DDAB [18] and in its mixtures [19] with sodium dodecyl sulfate (SDS). The SLS data in the Guinier plots (Fig. 1a)
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