HYALURONAN-SURFACTANT COLLOIDS FOR NANOMEDICAL APPLICATIONS

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Abstract

Hyaluronan as a negatively charged polyelectrolyte can easily interact with positively charged surfactants. Resulting complexes often phase separate in the form of precipitate. Phase separation can be controlled or suppressed by addition of a low molecular weight electrolyte or by a non-stoichiometric charge ratio. Resulting colloids combine hydrophilic biopolymer with hydrophobic domains capable of solubilizing non-polar substances. Due to hyaluronan biocompatibility and existence of specific cell receptors for hyaluronan such colloidal systems can find use in targeted delivery of water-insoluble drugs. This contribution discusses possibilities of preparing stable hyaluronan-surfactant colloids.

Keywords:
drug delivery, hyaluronan, surfactants

1. INTRODUCTION

Hyaluronan is a naturally occurring linear high molecular polysaccharide. It is a biopolymer with a wide range of naturally occurring molecular masses from several hundred to 10 million g.mol\(^{-1}\) possessing one carboxylate group per disaccharide repeating unit, and is therefore a polyelectrolyte bearing a negative charge. Hyaluronan can be found primarily in the extracellular matrix of all higher organisms, especially in connective tissues, synovial fluid, and eye vitreous and is produced by certain strains of bacteria. Hyaluronan is therefore an attractive building block for new biocompatible and biodegradable materials that could have applications in drug delivery, tissue engineering, wound healing, viscosupplementation or surgery [1]. Hyaluronan is highly hydrophilic biopolymer with massive hydration shell and cannot be directly used to carry nonpolar substances. Because many efficient drugs, e.g. for fighting cancer, are hydrophobic, hyaluronan should be modified in a way that enables formation of hydrophobic domains in aqueous hyaluronan colloids. A possible – “physical” – modification which is achieved by combination of hyaluronan with surfactant may lead to formation of associates in which the surfactant hydrophobic domains solubilize hydrophobes and hyaluronan plays a role of biocompatible carrier and targeting agent. Hyaluronan interactions with cationic surfactants were studied as a specific case of general polyelectrolyte-surfactant interactions to elucidate their phase behavior and physical causes of their interactions including the effect of electrolytes [2-4].

2. MATERIALS AND METHODS

Hyaluronan of bacterial origin at different molecular weights (see below) was received from CPN (Czech Republic). Cationic surfactants cetyltrimethylammonium bromide (CTAB) and tetradecyltrimethylammonium bromide (TTAB) of the best available purity were purchased from Sigma-Aldrich and used as received. Hyaluronan solutions were prepared by slow addition of powdered biopolymer into water upon stirring and left stirred for 24 hours to ensure complete dissolution. Hyaluronan-surfactant colloids were characterized by standard method of fluorescence probes using pyrene and calculating its emission polarity index (EmPI) [5]. EmPI indicates polarity of pyrene environment, the higher the EmPI value the more polar environment.
Turbidity was measured by UV-VIS spectrometer Cary 50 (Varian) equipped with fiber optics probe (optical path length 1 cm).

3. RESULTS AND DISCUSSION

Hyaluronan and cationic surfactant form complexes (aggregates or associates) which easily phase separate in water, especially at higher surfactant and/or hyaluronan concentrations. Example is shown in Fig. 1.

![Fig. 1. Phase behavior of TTAB in 0.1% (w/v) solution of high molecular weight hyaluronan (1700 MDa); TTAB concentration from left to right: 0.5; 0.75; 1; 2; 2.5; 5 mmol/l](image)

Type of colloidal dispersion changes from clear sols through turbid dispersion to phase separated system with precipitate and liquid supernatant. At sufficiently high surfactant concentration and at the present of small amount of low molecular weight electrolyte the precipitate changes to viscous gel upon standing during about one day. Complete precipitation in Fig. 1 occurs when surfactant:hyaluronan charge ratio is about 1, i.e. at charge neutralization.

Fluorescence probe experiments show that very small amount of micelles, hydrophobic domains in general, is formed in clear colloids of Fig. 1 and similar ones prepared from CTAB. Such systems are incapable of efficient solubilization of hydrophobic molecules. Decreasing hyaluronan concentration surfactant concentration region was found in which clear and stable sols with sufficient solubilizing power are formed. Behind this region (at higher surfactant concentrations) there is a narrow region of surfactant concentration in which small amount of gel phase separate gathering most of micelles with solubilized hydrophobic probes. Yet further behind, phase separation (precipitation) occurs. Before this region (at lower surfactant concentrations) no or very small amount of micelles are formed. This region was not revealed in previous studies on phase diagrams of hyaluronan-cationic surfactants systems [2-3].

Example of this “clear and stable” solubilizing region is shown in Fig. 2 and covers the interval of CTAB concentration between 0.03 and 0.1 mmol/l. This corresponds to surfactant:hyaluronan charge ratio between 1.2 and 40, respectively. It is supposed that surfactant micelles bound on hyaluronan chains (the “pearl necklace” structure) are formed in this region. Behind this interval a narrow interval of gel formation occurs which includes most of formed micelles. Adding more surfactant first dissolves the gel and finally leads to phase separation (precipitation).
Another possibility to prepare hyaluronan-micellar colloids is hyaluronan binding to preformed micelles. In this case, “concentrated” hyaluronan solution is progressively added to surfactant micellar solution. Depending on surfactant concentration clear or turbid colloids can be prepared the latter being stable in a broad interval of composition, i.e. not suffering from phase separation (precipitation). Thus adding high molecular weight hyaluronan (1730 MDa) to 10 mmol/l solution of CTAB resulted in progressive increase in turbidity as demonstrated in Fig. 3. Three regions of practically linear turbidity increase are indicated in the figure. Similar experiments with 3 mmol/l solution of CTAB gave only clear colloidal solutions within the same hyaluronan concentration range. Note that the critical micellar concentration of CTAB is about 0.9 mmol/l.

Adding NaCl at physiological concentration (0.15 mol/l) caused clarification of turbid systems. This is an indication of decreased particle size or even destruction of hyaluronan-micelle complexes. It is a matter of future investigations if increased ionic strength of dispersion phase really detaches micelles from hyaluronan chain by screening electrostatic interactions or if only decrease their size or change their arrangement. Calorimetric investigations indicate (data not shown here) that particularly in the case of CTAB which possesses rather long alkyl chain also hydrophobic or excluded volume effects play important role in its interactions with hyaluronan.
4. CONCLUSION
Phase behavior of hyaluronan-cationic surfactant systems is yet richer than revealed in previous experiments [2-4]. Stable clear colloids combining hydrophilic biopolymer with hydrophobic micellar domains can be formed in water at very low biopolymer concentration only and sufficient stoichiometric excess of the surfactant. Another possibility is to bound hyaluronan to surfactant micelles at concentration slightly above its critical micellar concentration.

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LITERATURE