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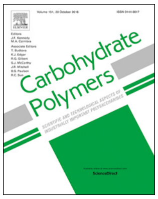

Ultrasonic study of hyaluronan interactions with Septonex—A pharmaceutical cationic surfactant

A. Kargerová, M. Pekař*

Brno University of Technology, Faculty of Chemistry, Materials Research Centre, Purkyňova 118, Brno, CZ-61200, Czech Republic

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ABSTRACT

Interactions between hyaluronan and the cationic surfactant Septonex were studied using high resolution ultrasound spectroscopy. Four different interaction regions were identified in aqueous solution in a narrow interval of surfactant concentrations. In contrast, in 0.15 mol/L NaCl solution, essentially only two principal regions were observed. Hyaluronan-Septonex aggregates were generally less compressible than Septonex monomers or micelles and their formation in water was not appreciably affected by hyaluronan molecular weight. In the presence of NaCl, the effect of hyaluronan molecular weight was more profound, which can be ascribed to its conformational sensitivity to ionic strength combined with its role as a surfactant counterion. Hyaluronan-Septonex interactions were closer to interactions with CTAB than with TTAB. Differences in structure or Krafft temperature (solubility) between Septonex and CTAB were thus not significantly reflected in these interactions. The basic characteristics of Septonex surfactant were also determined.

1. Introduction

Hyaluronan is a well-known biopolymer synthesized in various organisms including the human body. It is formed by a repeated basic dimeric unit composed of glucuronic acid and N-acetyl-glucosamine. Despite its simple structure, hyaluronan manifests a variety of physiological functions which are the consequences of its molecular weight, interactions with water (the hydration shell), ions, and other components of biological environments including cell membranes. It usually occurs as a sodium salt and the name hyaluronan should be understood in this way here. Due to its biological origin, biocompatibility, non-immunogenicity, and biodegradability, hyaluronan has found various applications in the fields of cosmetics and medicine.

Due to the presence of carboxylic groups in its backbone, hyaluronan behaves in aqueous environments as a polyelectrolyte. At physiological pH values, hyaluronan possesses negative charge and is thus capable of attractive electrostatic interactions with positively charged partners. Thus, interactions between hyaluronan and cationic surfactants have been intensively studied as a specific example of a broader class of polyelectrolyte-surfactant interactions. The binding of surfactant to hyaluronan was detected for surfactants with an alkyl chain consisting of at least ten carbon atoms (Bjoerling, Hersloef-Bjoerling, & Stilbs, 1995; Hersloef, Sundeloef, & Edsman, 1992; Thalberg & Lindman, 1989; Thalberg, Lindman, & Karlstroem, 1990). Phase separation was observed as the formation of a precipitate or a gel-like phase upon increasing surfactant concentration – the longer the surfactant alkyl chain, the larger the size of the phase separation region. Due to the prevailing electrostatic nature of hyaluronan-cationic surfactant interactions, the addition of an electrolyte, e.g. NaBr, suppressed phase separation, which disappeared at 250 mM NaBr, whereas another type of phase separation was observed with NaBr concentrations at and above 500 mM (Thalberg, Lindman, & Karlstroem, 1991). Such published results indicate the strong cooperativity of surfactant binding on the hyaluronan macromolecule, resulting in binding in the form of micelle-like clusters in which both hyaluronan carboxylates and background electrolyte anions participate as counterions.

Recently (Kargerová & Pekař, 2014b), we demonstrated that high resolution ultrasound spectroscopy can bring new information on the dynamics of polyelectrolyte-surfactant interactions (seen during titration experiments) not accessible by, for example, isothermal titration calorimetry, which is routinely used in this field (Bao, Li, Gan, & Zhang, 2008; Lapitsky, Parikh, & Kaler, 2007). Surprisingly, ultrasound spectrometry is rarely used to study polyelectrolyte-surfactant interactions. Perhaps the only detailed study (La Mesa, Persi, & D´Aprano, 1998) reported on interactions between anionic sodium dodecylsulphate and (nonionic) polyethylene oxide or (zwitterionic, in fact) polyvinylpyrrolidone determined by measurements of ultrasound velocity and compressibility. Data for the polyethylene oxide-containing system

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* Corresponding author.
E-mail address: pekar@fch.vut.cz (M. Pekař).

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were rather scattered with no clear trends, which was explained by the absence of (electrostatic) interactions between the neutral polymer and charged surfactant. The authors focused their discussion mainly on data for the polyvinylpyrrolidone-containing system, which are plotted and discussed in Figure SF1 in Supplementary Information. Buckin, Kudryashow, Morrissey, Kapustina, and Dawson, (1998) investigated interactions between DNA and alkyltrimethylammonium bromides using high-precision ultrasonic velocity measurements and concluded that micelle-like surfactant aggregates are formed on the DNA surface. Another application of the ultrasound technique can be found in the study by Gonzáles-Gaitano, Crespo, and Tardajos, (2000) of systems containing β-cyclodextrin, which, of course, is not a typical polyelectrolyte, and alkyltrimethylammonium bromides. Further knowledge on the application of ultrasound to polyelectrolyte-surfactant systems is thus desirable, also from the methodological point of view and for evaluating the potential of ultrasound techniques in this field.

High resolution ultrasound spectrometry revealed the existence of several transitions during the titration of hyaluronan with the cationic surfactants tetradecyl- and hexadecyl-trimethylammonium bromides (TTAB and CTAB, respectively) (Kargerová & Pekař, 2014b). The transitions were dependent on the length of the surfactant alkyl chain and marked differences were observed between TTAB and CTAB. In this work, we were interested in the behavior of a structural analogue of these two surfactants – carbethoxypendecinium bromide, produced under the trade name Septonex. Septonex possesses a pentadecyl alkyl chain and an ethoxycarbonyl group in the vicinity of the trimethylammonium group (the polar head; see Fig. 1). Thus, it is of interest to see how these relatively small structural changes affect the interactions to hyaluronan. Septonex was chosen also because it is used as a constituent of several pharmaceutical products mainly due to its antimicrobial activity – for example, skin disinfection liquids, wound-treating sprays, eye drops, and ointments to fight eye infections and inflammation. In fact, aggregates between hyaluronan and Septonex (cationic surfactants in general) are not only of general interest but can be interesting with respect to delivery applications for hydrophobic active agents (drugs). Surfactant micelles bound on native hyaluronan can solubilize hydrophobic molecules while being protected, transported, and targeted by the biopolymer chain.

2. Materials and methods

2.1. Materials

Hyaluronan of several molecular weights, extracted from the cell walls of the bacteria *Streptococcus zooepidemicus*, was obtained from Contipro Biotech (Czech Republic). This producer offers a broad range of molecular weights in a predefined range of molecular weights. The following hyaluronan products were used in this study: 10–30 kDa, 90–130 kDa, 300–500 kDa and 1500–1750 kDa; the concrete molecular weights of particular samples from each range used in this study (determined by the producer using SEC-MALS) are given in Table ST1 in Supplementary Information. Septonex of pharmaceutical quality was purchased from GBNchem (Czech Republic) and sodium chloride of 99.5% purity was obtained from Lachner (Czech Republic).

Hyaluronan solutions were prepared at a concentration of 1000 mg/l (w_0) by dissolving hyaluronan powder in water or in 0.15 M NaCl. The solution was stirred for 48 h at room temperature to ensure complete dissolution. Ultrapure deionized water from a PURELAB (Option R7/15; ELGA, Great Britain) water purification system was used for the preparation of all samples.

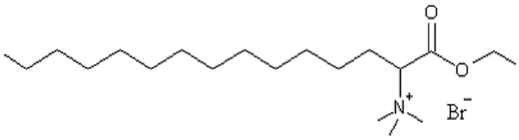

Fig. 1. Structural formula of Septonex.

2.2. Methods

Ultrasound velocity and attenuation were measured at seven selected frequencies in the range from 2.5 to 17.5 MHz using an HR-US 102 T ultrasonic spectrometer (Sonas Technologies, Ireland) with titration accessory. Details can be found in Supplementary Information. Briefly, the measuring cell was filled with deeply degassed hyaluronan solution and the Septonex solution was added through the titration accessory. The reference cell was filled with ultrapure and degassed deionized water. Ultrasonic velocity and attenuation were monitored continuously over the course of the titration at 25 °C.

Each measurement was made at least in duplicate, and average values are reported in figures for the sake of clarity. Reproducibility was much better in the case of velocity than attenuation, as expected. Examples of velocity data with standard deviations are given in Figure SF2 in Supplementary Information. The highest deviations were found in the phase separation region (see below); otherwise, the deviation was on average below about 10%. Attenuation was measured with much less precision and an example is also given in Supplementary Information (Fig. SF3). This did not affect the conclusions of this work because attenuation was used only as an auxiliary qualitative parameter and only the quite reproducible shapes of the measured attenuation curves were relevant. The effect of ultrasound frequency on the measured titration curves and the parameters determined from them was negligible (cf. Fig. SF4 in Supplementary Information) and thus only single frequency data are reported here.

The critical micellar concentration is an important parameter of any surfactant. Data on the critical micellar concentration of Septonex are rather scarce and published works used (only) surface tension, conductivity, or UV-absorbance techniques. The first step of our work was thus the determination of this value using high-resolution ultrasound spectroscopy in order to verify the technique and to compare its outputs with published data, and to use the results obtained with pure surfactant as a basis for the interpretation of results obtained for systems with hyaluronan. Measurements were realized in pure water and in 0.15 M NaCl solution.

The surfactant/hyaluronan charge ratio was calculated assuming the presence of one negative charge on each hyaluronan disaccharide unit; Septonex was assumed to carry one positive charge on its molecule. The concentration of hyaluronan disaccharide units (in mol/L) was calculated from the (sodium) hyaluronan concentration (in g/L) and molecular weight, and the disaccharide repeating unit molecular weight (sodium form; 401.299 g/mol).

Ultrasonic measurements are usually accompanied by measurements of density in order to enable calculations of compressibility from ultrasonic velocity and density. A DSA 5000 M densitometer (Anton Paar, Austria) was used in this work; for details see Supplementary Information. The density (ρ) and the ultrasound velocity (U) were used to calculate the (adiabatic) compressibility (β) according to the well-known equation $\beta = 1/(\rho U^2)$ (sometimes called the Laplace or Newton-Laplace equation).

3. Results and discussion

A high-resolution ultrasound spectrometer gives, as its output, the difference in velocity or attenuation measured between the sample and reference cells, i.e. in our case, the difference between the value in the sample and the values in water or NaCl solution. Data are therefore reported in terms of velocity difference (dU) and attenuation difference (dN).