EXCITED-STATE PROTON TRANSFER STUDY IN POTENTIAL HYALURONAN-SURFACTANT DRUG NANOCARRIERS

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Abstract

Hyaluronan is a biopolymer suitable as a targeting unit in drug delivery systems according to affinity of hyaluronan to CD-44 receptor of cancer cells. We suppose that hyaluronan, when interacts with cationic surfactant, influences hydration of Stern layer of CTAB which affects fluorescence properties of 1-naphthol. We study excited state proton transfer of 1-naphthol in aqueous solution and in hyaluronan-cetyltrimethylammonium bromide (CTAB) system by steady-state and time-resolved fluorescence techniques. Neutral naphthol form shows change in fluorescence intensity of aqueous micelle solution around reported critical micelle concentration (~1 mM) which suggests solubilisation of 1-naphthol in micelles. Steady-state study of neutral naphthol in hyaluronan-CTAB system shows similar behaviour as in aqueous CTAB system. On the contrary the time-resolved study of 1-naphthol shows change in fluorescence lifetimes of neutral and anion naphthol forms with increasing CTAB concentration. The change in fluorescence lifetimes of anion naphthol before critical micelle concentration suggests formation of hyaluronan-CTAB aggregates before critical micelle concentration is reached. Kinetic study of excited state proton transfer showed that hyaluronan influenced proton transfer kinetics of 1-naphthol and suggested that hyaluronan influenced Stern layer of micelle.

Keywords: Hyaluronan, excited state proton transfer, hydration

1. INTRODUCTION

Hyaluronan-surfactant (liposome) interactions provide useful model for anticancer drug delivery system based on hyaluronan sensitivity for CD44 receptor.[1],[2],[3] Hyaluronan is a biocompatible and biodegradable polysaccharide consisting of disaccharide units (D-glucuronic acid and N-acetyl-D-glucosamine (Chyba! Nenalezen zdroj odkažu.).[4] Delivery systems based on hyaluronan are known to be quite selective for tumor cells because CD44 receptor is overexpressed in cancer cells.[5] Micelle likely provides, in this model, good environment for drug solubilization, stabilization or controlled release and hyaluronan provides controlled targeting of the drug. Hyaluronan in aqueous solution forms specific overlapping domains which causes high water-retention capacity. The hydration or water-retention capacity is probably one of the most important aspects of the hyaluronan biological functions.[6],[7] The hyaluronan-cationic surfactant interaction have been described in many previous papers[8],[9],[10],[11],[12] but no reports about influence of hyaluronan hydration in hyaluronan-surfactant interaction have been reported to date.

Excited state proton transfer of aromatic dyes has been widely studied in bulk water or systems such as micelles[13],[14],[15], proteins[16],[17], reverse micelles[18], cyclodextrins[19],[20], nafions[21] or polymer-surfactant interactions[22],[23],[24]. Excited state proton transfer fluorescence probe 1-naphthol exhibits dual fluorescence with emission peaks of neutral form (NpOH) at ~350 nm and emission peak of anion form (NpO-) at ~450 nm. The emission of NpO- is dominant in water environment and NpOH emission is dominant in hydrophobic environment or in alcohols[25],[26]. In bulk water, naphthol undergoes proton transfer in 35 ps, which causes extremely low fluorescence intensity of neutral naphthol form. When 1-naphthol is solubilized into micelles, proton transfer rate is retarded and neutral fluorescence intensity is seriously increased.[13] When ESPT fluorescence probe adsorbs to polymer, proton transfer rate changes as well as fluorescence intensity of its emission peaks. This phenomenon was used to study hydration of some polymers such as poly N-vinyl-2-pyrrolidone[27] or polyvinyl alcohol.[28] Spry et al used proton transfer dynamics as a tool to study
water environment in nafion fuel cell membranes and anionic surfactant aerosol OT in heptane as a solvent. The rate of proton transfer differed with size of nafion or aerosol OT. The proton transfer was influenced by change of hydrogen bonding network of water as the water pools are reduced in size which got fluorescence probe closer to nonpolar solvent.[21] Das et al studied proton transfer dynamics in anionic, cationic and nonionic micelles. Their results suggests that proton transfer in cationic micelles is the slowest one because –N(CH3)₃⁺ headgroups inhibit the approach of water molecules close to the Stern layer of cationic micelles.[29]

The aim of this work is to report about the study of hyaluronan-CTAB interactions by excited state proton transfer and the environmental change of CTABs Stern layer due to hyaluronan hydration. The extreme sensitivity of ESPT to presence of water molecules makes fluorescence study of ESPT convenient to assess polymer-surfactant interactions. Herein, we present steady-state and time-resolved fluorescence study of 1-naphthol in above mentioned system as well as report of dynamic study of processes involved in excited state proton transfer of 1-naphthol in hyaluronan-CTAB system.

2. RESULTS AND DISCUSSION

Fig. 1 shows steady-state emission spectra of 1-naphthol in water, hyaluronan, CTAB, hyaluronan-CTAB and water-methanol mixtures. The fluorescence intensity ratio of neutral and anion naphthol in water is 1:42. Addition of 2 mM CTAB increased fluorescence intensity of anion emission with concomitant increase of neutral emission peak. The NpOH:NpO⁻ intensity ratio changed to 1:16. This intensity ratio is significantly smaller than in bulk water which would appear that significant hindrance of excited-state proton transfer reaction occurred due to naphthol molecules solubilisation into micelles. As described by Kim et al, 30-50 molecules of water are needed to promote ESPT reaction.[30] Fluorescence spectrum of 1-naphthol in CTAB shows major peak at 450 nm. In hydrophobic environment emission of anion naphthol would be significantly hindered, because there are not enough water molecules around naphthol to promote ESPT. Our observation suggests that naphthol is located in palisade layer of CTAB micelles and fluorescence spectrum is influenced by hydration of Stern layer. On the other hand the proton transfer was most effective in hyaluronan system. The NpOH:NpO⁻ ratio in hyaluronan increased to 1:56. The fluorescence intensity ratio in hyaluronan is significantly different from that of bulk water. The increase in proton transfer efficiency suggests adsorption of naphthol in hyaluronan bound water region as similarly reported for poly (N-vinyl-2-pyrrolidone) (PVP).[27] As comparison of effective hindrance of ESPT is depicted the fluorescence spectrum of 1-naphthol in 80 % water-methanol mixture. Ratio of fluorescence bands of neutral and anion naphthol form is 1:0.9 suggesting significant hindrance of proton transfer in this system. The retardation of proton transfer by presence of alcohols is described elsewhere.[26]

![Fig. 1 Steady-state fluorescence emission spectra of 1-naphthol in water (---), 7 mg/L hyaluronan (●●●), 2 mM CTAB (-●-), 0,5 mM CTAB, 7 mg/L hyaluronan (— — —), 2 mM CTAB and 7 mg/L hyaluronan (— — ) and 80 % methanol-water mixture (—— —). The inset depicts the range of neutral naphthol emission](image-url)
Steady-state emission intensity of both neutral and anion naphthol increased in 7 mg/L hyaluronan solution after addition of 2 mM CTAB. The NpOH:NpO⁻ ratio decreased to 1:15, which suggests solubilisation of naphthol into micelles as well as in case of 2 mM CTAB in absence of hyaluronan. When 0.5 mM CTAB was added to 7 mg/L hyaluronan solution, NpOH:NpO⁻ fluorescence intensity ratio decreased to 1:45 when compared to hyaluronan with absence of CTAB which represents the water like ratio. According to Thalberg and Lindman, the electrostatic interaction between hyaluronan and CTAB forms hyaluronan-CTAB aggregates before critical micelle concentration of CTAB (~1 mM).[9] The 5 nm shift in emission wavelength maximum is observed which indicates solubilisation of naphthol to CTAB aggregates (Chyba! Nenalezen zdroj odkazů.), but no significant increase of NpOH fluorescence intensity occurs. When titration of naphthol, naphthol-hyaluronan and pyrene-hyaluronan system with CTAB was compared, no significant change in comparison between naphthol and naphthol-hyaluronan system is observed (Fig. 2) and from the plot of NpOH fluorescence intensity as function of CTAB concentration it seems that no hyaluronan-CTAB aggregates are formed before critical micelle concentration of CTAB. Comparison of plot of excitation polarity index of pyrene against CTAB concentration indicates formation of CTAB-hyaluronan aggregates at 0.05 mM concentration (critical aggregation concentration) and CMC would appear to decrease to 0.5 mM. The comparison of normalized NpOH and NpO⁻ fluorescence intensities as function of CTAB concentration shows difference between both plots (Fig. 3). NpO⁻ fluorescence intensity increases around 0.02 mM followed by repeated decrease of fluorescence intensity. The second increase occurs at 0.5 mM concentration of CTAB. The dependence of anion fluorescence intensity on CTAB concentration from Fig. 2 correlates with results of titration of pyrene-hyaluronan system from Fig. 1. Fluorescence intensity of NpOH form should differ as well as NpO⁻ emission when aggregation occurs but the neutral naphthol emission is probably influenced by higher representation of water molecules in micellar environment. Naphthol in hyaluronan-CTAB aggregates is likely to be affected by higher hydration of micelles Stern layer. In the following section is discussed the effect of hyaluronan on fluorescence decay of naphthol in hyaluronan-CTAB aggregates.

Fig. 2 Titration of naphthol (○), pyrene-hyaluronan (Δ), naphthol-hyaluronan (◊) and naphthol-PSS (x) system with CTAB
Fluorescence decay of NpOH was always fitted with triexponential function. Fluorescence lifetimes are summarized in Chyba! Nenalezen zdroj odkazů. Decay of neutral naphthol in presence of hyaluronan consists of fast time of deprotonation and two slower times of 1362 ps and 7880 ps (Chyba! Nenalezen zdroj odkazů.). Fast component is 1.5 times higher than in bulk water, the first slow component is water-like component and the second slow component represents decay of NpOH. The fluorescence decay suggests influence by adsorption of naphthol to hyaluronan, because the deprotonation rate is influenced by polymer bound water, not by bulk water. Also the average fluorescence lifetime increased two times in presence of hyaluronan which is as well as in case of PVP caused by adsorption of probe to polymer bound water.[27] The significant change in relative amplitudes, when compared to water, of two slow decay components also suggests that fluorescence decay of NpOH does not belongs to naphthol in bulk water but to adsorbed naphthol. NpO⁻ decay in presence of hyaluronan was fitted with biexponential function with bulk water like rise time of 1515 ps and decay time of 8052. Adsorption of naphthol to hyaluronan bound water does not affect NpO⁻ decay, because relative amplitudes of NpO⁻ in water and in presence of hyaluronan have comparable lifetimes and relative amplitudes.

The NpO⁻ decay in 0.05 mM CTAB and presence of hyaluronan was fitted with triexponential function with two rise times of 1270 ps and 8074 ps and decay time of 20 ns. The increase in NpO⁻ decay time may be ascribed to decay from hyaluronan-CTAB aggregates. The shift of critical micelle concentration from 1 mM down to 0.5 mM (see higher in the text) is similarily shown also in Chyba! Nenalezen zdroj odkazů. Comparison of NpO⁻ decay parameters of 0.5 mM CTAB in absence and presence of hyaluronan shows seven fold increase in rise time and increase in decay time to 17 ns in presence of hyaluronan. Again the decay time of naphthol form may be represented by solubilisation in hyaluronan-CTAB aggregates. The comparison of fluorescence decay curves of NpO⁻ is shown in Chyba! Nenalezen zdroj odkazů.a. In hyaluronan-CTAB aggregates ([CTAB] = 0.05 mM) the fluorescence decay of neutral naphthol exhibits comparable decay parameters to NpOH decay in absence of surfactant. Fluorescence lifetimes and relative amplitudes would appear to be hyaluronan like components when compared to 2 mM CTAB in presence of hyaluronan. Comparison of fluorescence decay curves of NpOH are displayed in Chyba! Nenalezen zdroj odkazů.b. As described higher in the text, NpO⁻ decay parameters are not influenced by polymer bound water, thus NpO⁻ fluorescence lifetimes may indicate naphthol solubilisation. From the discussion above the results indicate that decay of NpOH is probably influenced by hydration shell of hyaluronan. Steady-state experiments suggest naphthol solubilisation in Stern layer of CTAB micelle where. When hyaluronan-CTAB aggregates are formed, hyaluronan hydration shell probably reaches to Stern layer of micelle and changes fluorescence lifetimes of neutral naphthol. The influence of hyaluronan hydration seems to be observed also in concentration of CTAB after CMC (2 mM). The lifetimes of NpOH in absence and presence of hyaluronan are comparable but major
component in presence of hyaluronan became the fast component of 337 ps which belongs to rate constant of deprotonation and this phenomenon may be ascribed to change of the environment of Stern layer. The rate of deprotonation in 2 mM micelle solution is almost twelve times hindered than in bulk water due to naphthol solubilisation. At 0.05 mM concentration, kinetic study shows retardation of proton transfer when compared to bulk water approximately by factor of two. At 0.5 mM CTAB concentration hyaluronan-CTAB system shows higher deprotonation rate constant than at 0.05 mM concentration. The rate of deprotonation in hyaluronan-CTAB aggregates probably slows down when critical micelle concentration is exceeded maybe due to formation of larger aggregates where hyaluronan hydration has not such influence as in CTAB concentration before CMC. The deprotonation rate in 2 mM CTAB in presence of hyaluronan shows almost two times faster deprotonation rate than in micelles or methanol which suggests the influence of hyaluronan hydration in Stern layer of CTAB micelles.

3. CONCLUSIONS

ESPT dynamics is extremely sensitive to water environment. In water, ESPT of 1-naphthol is extremely fast which correlates with extremely low fluorescence intensity of neutral form of naphthol. When 1-naphthol solubilizes in micelles, rate of deprotonation is retarded with concomitant increase in fluorescence intensity of NpOH. The solubilisation of naphthol occurs also in hyaluronan-CTAB system when surfactant concentration is before critical micelle concentration, but the proton transfer rate is much faster than in micelles with absence of polymer. The pyrene study correlates with assumption that hyaluronan-CTAB aggregates are formed before critical micelle concentration and time-resolved and proton transfer dynamic studies show the difference between environment in hyaluronan-CTAB and CTAB micelles.

ACKNOWLEDGMENTS

This work has been supported by the project “Centre for Materials Research at Brno University of Technology, Faculty of Chemistry” No. CZ.1.05/2.1.00/01.0012 from ERDF.

LITERATURE


